Weed Science

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New Approaches to Herbicide and Bioherbicide Discovery

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Abstract

During the past 30 yr an impasse has developed in the discovery and commercialization of synthetic herbicides with new molecular targets and novel chemistries. Similarly, there has been little success with bioherbicides, both microbial and chemical. These bioherbicides are needed to combat fast-growing herbicide resistance and to fulfill the need for more environmentally and toxicologically safe herbicides. In response to this substantial and growing opportunity, numerous start-up companies are utilizing novel approaches to provide new tools for weed management. These diverse new tools broaden the scope of discovery, encompassing advanced computational, bioinformatic, and imaging platforms; plant genome–editing and targeted protein degradation technologies; and machine learning and artificial intelligence (AI)-based strategies. This review contains summaries of the presentations of 10 such companies that took part in a symposium held at the WSSA annual meeting in 2024. Four of the companies are developing microbial bioherbicides or natural product–based herbicides, and the other six are using advanced technologies, such as AI, to accelerate the discovery of herbicides with novel molecular target sites or to develop non-GMO, herbicide-resistant crops.

Introduction

Few herbicides with new molecular targets have been introduced in the past four decades (Duke and Dayan 2021). Before this drought in new modes of action (MOAs), a herbicide with a new MOA was introduced approximately every 3 yr (Gerwick 2010). The last significant new MOA (hydroxyphenylpyruvate dioxygenase) was introduced in the 1980s. Since then, only two new MOAs (homogentisate solanesyltransferase and dihydroorotate dehydrogenase) from newly introduced herbicides have been added to the Herbicide Resistance Action Committee MOA classification scheme (HRAC 2024), represented by only two herbicides (cyclopyrimorate and tetflupyrolimet, respectively) (Kang et al. 2023; Shino et al. 2020), one of which (tetflupyrolimet) is not yet commercially available. Other new MOAs have been added to the HRAC classification scheme in recent years, but these have been the result of discovery of the MOAs of older herbicides for which the MOA was previously unknown, for example, proof that the MOA of endothall is the inhibition of serine/threonine protein phosphatase (Bajsa et al. 2012).

Due to the lack of novel classes of herbicides, the market is currently driven by a relatively small number of relatively old molecules. Moreover, some herbicides with MOAs discovered decades ago are increasingly being called into question because of environmental and toxicological issues. Consequently, the challenge is to go beyond developing analogues of chemical families with existing MOAs and find an entirely new generation of herbicides with novel MOAs. The question is how can a whole new generation of herbicides working in novel ways be found to tackle the drought in novel MOA discovery?

Review

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The evolution and spread of herbicide resistance have grown rapidly during the past few decades (Heap 2024), increasing the need for herbicides with new chemistries to fight non–target site resistance and with new molecular targets to counter target-site resistance. Without effective herbicides, farmers can lose up to 40% of their crop yields from weed interference (Duke and Dayan 2021; Nickel and Polansek 2024). The reasons for the dramatic decline in the introduction of much-needed herbicides with new MOAs are several, as discussed in detail by Duke and Dayan (2021) and Powles (2023). One of the explanations for the dramatic decline in new herbicides is the diminishing returns with traditional chemical synthesis and screening of potentially herbicidal compounds. Similarly, in addition to the paucity of new herbicides with new MOAs, the introduction of significantly successful bioherbicides (both microbial bioherbicides and natural product–based herbicides) has been at a standstill for decades for different reasons than those of the attrition in introduction of new synthetic herbicides (Duke 2024).

In the past decade, numerous small (start-up) companies have been formed that offer different approaches to herbicide and bioherbicide discovery (Dayan 2019). Powles (2023) mentions a dozen of these companies in a recent review, but there is no detailed information about their novel approaches to herbicide discovery or any of their discoveries. The present review is based on presentations from the "'New Approaches to Herbicide and Bioherbicide Discovery" symposium organized during the 64th Annual Conference of the Weed Science Society of America (WSSA) held in San Antonio, TX. This symposium brought together speakers from 10 such companies with new approaches to herbicide and bioherbicide discovery. Summaries of their presentations are provided in the following sections.

Biocontrol and Natural Product–Oriented Approaches

Biocontrol of weeds in crops with microbes has largely been unsuccessful, despite many patents, products, and start-up companies over the past 40 yr. Duke (2024) and Gressel (2024) discuss the many technical reasons for this impasse in their recent analyses of the status of microbial bioherbicides, and Marrone (2024) discusses the lack of progress in the area of bioherbicides in the context of other categories of biopesticides and the pesticide market in general. The first paper in this section describes a promising new approach to biocontrol of striga [*Striga hermonthica* (Delile) Benth.] with a genetically selected mycoherbicide (Baker et al. 2024; Lüth et al. 2024). The start-up company (Toothpick Company) with this product was chosen for the 2024 Sankalp Africa Award, an award for outstanding social entrepreneurs. The next section from ProFarm Group discusses its work with microbially produced phytotoxins with novel MOAs. Although these products could be considered microbial bioherbicides under one U.S. Environmental Protection Agency (USEPA) definition of such products (Duke 2024), their activity depends primarily on strong phytotoxins with novel molecular targets such as spliceostatin C (Bajsa-Hirschel et al. 2023) produced by the microbes.

Natural compounds have provided leads for new commercialized insecticides and fungicides with new molecular targets, but less so for herbicides (Sparks et al. 2023). However, there are many highly phytotoxic natural compounds with novel molecular targets that have not been fully explored for herbicide use (Dayan and Duke 2014). In the third part of this section, INBIOAR Global discusses approaches for discovery of novel phytochemical phytotoxins with promise for use in crude extracts or as leads for novel synthetic herbicides (Sosa et al. 2021). The final part of this section discusses an approach by MicroMGx of using systematic analysis of biosynthetic gene clusters (BGCs) and metabolomics data for discovery of novel herbicidal compounds from microbes. Recent success in finding a herbicidally potent natural phosphonate is described.

Kichawi Kill™, a Maize Seed–Coating Technology Delivering Virulence-selected Fusarium for Striga Biocontrol

Herbicide resistance and concerns about the toxicity of synthetic herbicides are two of the reasons for developing bioherbicides in high-income countries such as the United States. However, in Kenya in 2007, an unusual set of reasons were presented regarding *S. hermonthica*, a widespread invasive, parasitic weed across sub-Saharan Africa. Farmers losing 20% to 100% of their crop yield due to this parasitic weed struggled with a range of conditions that prevented their ability to it. These conditions included the lack of synthetic chemical herbicides available at the village level, farmers' concerns about the potential human toxicity of herbicides due to hand planting, and a lack of available funding for farm inputs. With most farmers working a hectare or less, they were seemingly overlooked by the primary input companies, and few local input distributors existed at the time.

The challenges surrounding striga demanded a new approach—one that was safe, affordable, effective, and, ideally, not committed to the introduction of problematic synthetic chemical herbicides. The use of a host-specific, endemic fungal pathogen for striga control was considered as an alternative. As defined by Gressel (2024), the four pillars for successful mycoherbicide commercial development are: (1) must have enhanced virulence; (2) must be cost-effectiveness; (3) shelf life must accommodates distribution; and (4) and must be biosafe without toxic impact beyond the target pest. Previous researchers were unable to achieve significant commercial success using endemic bioherbicides. Our hypothesis was that endemic, wild-type host-specific fungi would not be virulent enough to act as an effective bioherbicide. As noted by Ejeta and Gressel (2007), mortality against your only host is not a good evolutionary strategy. Given this situation, could a host-specific pathogen with higher virulence could be selected so that it could effectively, economically, and sustainably control its target host?

There have been many attempts to use pathogens that produced phytotoxins, and Toothpick's approach aims to avoid this approach to biocontrol of weeds in that it might be merely replacing one phytotoxin with another. The work of Steinberg (1952) interested us, because he demonstrated that essential amino acids were inhibitory to tobacco (*Nicotiana tabacum* L.) and that this seemed to be the MOA of two common soil-borne bacteria causing the frenching disease of tobacco. This approach appears to have important implications for its broader use in biocontrol of weeds, as most weeds surveyed so far, in fact most plants, are inhibited by at least one essential amino acid at some concentration. Given this knowledge, amino acid analogue selection was used to obtain plant pathogens that excreted select amino acids. These selections resulted in strains of *Fusarium oxysporum* f. sp. with enhanced virulence (Nzioki et al. 2016). While striga is inhibited by three amino acids, leucine, tyrosine, and threonine, maize (*Zea mays* L.) was tolerant to leucine and tyrosine, but sensitive to threonine; therefore, *F. oxysporum* strains excreting leucine and/or tyrosine were selected. Peacock and Muirhead found that methionine was converted to ethylene by soil microbes, and that it served as a general stimulator of soil seedbank germination (Peacock and Muirhead 1974). For this reason,

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Figure 1. (A) Untreated striga-infested field on Kisumu-Busia Road, Busia County, Kenya. (B) Striga-infested field treated with Kichawi Kill™ on Kisumu-Busia Road, Busia County, Kenya. Photos: Geoffrey Wanjala, Farm to Market Alliance, World Food Program.

F. oxysporum strains that were triple excreters of leucine, tyrosine, and methionine were selected to target striga and its soil seedbank.

Delivery of the selected fungal strains to farmers in rural Kenya was the next challenge. Striga seed germination is triggered by hormones emitted by the host-crop seed germination, allowing the hemiparasite to germinate and attach to the crop within 72 h. Our inclination was that a live, fresh inoculum was needed to inhibit the fast-germinating and growing weed. These selected strains of *Fusarium hermonthica* f. sp. *strigae* could be grown on toothpicks and distributed to farmers as primary inoculum. The farmers could then grow their own fresh secondary inoculum by placing the toothpicks in cooked rice to cultivate the fungus over 3 d. The farmer could then co-plant a pinch of this new inoculum in each planting hole with their seeds. This approach was successful, improving crop yield by 42% to 56% in 500 paired-plot trials over two seasons and demonstrating similar restorative outcomes in years of trials and farmer reports (Nzioki et al. 2016; Figure 1).

Through the social enterprise, Toothpick Company Ltd., the product received regulatory approval for commercial use in 2021. However, while effective in the field, the product had some issues, including a 10-d shelf life, risk of contamination, and the high cost for the rice substrate. Growing the fungal strains on a wood powder rather than toothpicks meant the powder could be used as a seed coating. This seed coating demonstrated efficacy in the field and received regulatory approval in June 2023 (Lüth et al. 2024). This iteration reduced the price by 60% and increased the shelf life to 3 mo at room temperature and a year in the freezer. This shelf stability allows new distribution channels, including through agrovet shops and distribution companies. In both internal surveys and studies conducted by a large distribution company, farmers are reporting average yield increases of 12.3 to 14.8 90-kg bags ha^{-1}. Overcoming the striga barrier results in economic growth for the household, and because the product is manufactured locally in western Kenya rather than imported, it also boosts the local economy. The details of the obstacles to commercialization of this product are provided by Baker et al. (2024).

The next steps for the Toothpick Project are to expand across sub-Saharan Africa, starting with Uganda, Ethiopia, and Cameroon. Regulatory protocol for biocontrol registration for commercial use varies from country to country. However, this is a growing area of regulatory development, with harmonized protocols emerging in the East African Community, for example. With this breakthrough in bioherbicide development, and keeping in mind Gressel's four pillars necessary for successful bioherbicide development, it is worth noting that other species of weed pathogens might also be useful in biocontrol of weeds.

The Toothpick Project is an example of how a scientifically sound bioherbicide technology can be developed to match the needs of farmers. In 2007 in Kenya, the need was urgent and growing, but more recent awareness of threats within the chemical herbicide industry (litigation due to human and environmental toxicity and herbicide resistance of hundreds of weed species) has brought awareness of the need for more sustainable approaches to weed control globally across the range of agricultural sectors.

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Kichawi Kill™ represents one such host-specific bioherbicide selected for enhanced virulence.

ProFarm Strategy to Overcome the Challenges of Developing Microbial Bioherbicides

In seeking to develop a bioherbicide, ProFarm scientists have found it useful to distinguish between three paradigms, each operating in largely separate niches. Burn-through products include many organic, naturally sourced extracts, acids, soaps, and oils that rapidly compromise cuticular integrity at the point of contact. These products are most often sold in the home and garden markets. Regrowth from plant parts not directly exposed, especially roots, is a problem with these products. A second category of bioherbicides controls weeds by infecting them with a live pathogen; these products are necessarily highly specific in their spectrum of activity, they may operate relatively slowly and over long-term time frames, and they can be highly suited to situations that require targeting an invasive or parasitic weed species without damaging the surrounding flora. Because these pathogen-based products use live organisms, they face certain commercial and supply-chain disadvantages in fitting into the row-crop or large-scale agricultural markets (Duke 2024; Duke et al. 2022) and are primarily developed for use on public lands by government agencies. By contrast, focusing on the deployment of natural product chemistry produced by plants or microbes can help a bioherbicide transcend some of the limitations imposed when a product relies on the action of live organisms or naturally sourced, burn-through extracts. In the case of microbial bioherbicides, once a fermentation cycle is complete and the desired metabolites have been produced, the microbes themselves can be inactivated, and the dead cells either included or removed from the final formulated product, thus negating the issue of spread of the metabolite-producing microbe to unintended plant species. Perhaps more importantly for this third category of bioherbicides, natural products can operate and be handled as phytotoxins, much like most conventional herbicides.

There are analogous examples of these bioherbicide paradigms in the biofungicide (FRAC 2023) and bioinsecticide (IRAC 2024) sectors, as well as many synthetically produced compounds that have been derived from natural products isolated from plants or microbes. In contrast to the insecticide and fungicide crop protection segments, there are hardly any examples of natural product–based or natural product–derived molecules available for control of weeds, with glufosinate—first isolated from several *Streptomyces* soil bacteria species—being the prominent exception. After decades of many well-conceived scientific efforts to develop a bioherbicide, it was posited that the persistent dearth of such products in the market may be due as much to technological and commercial challenges as it is to scientific challenges. ProFarm Group, a subsidiary of Bioceres Crop Solutions, is working to overcome some of these challenges in a pair of bioherbicide projects, each based on herbicidal compounds produced by soil-borne microbes. One is a project based on thaxtomin A (Figure 2), a molecule that belongs to Group 29 in the HRAC MOA classification system and is produced by the bacteria *Streptomyces acidiscabies*, which causes potato scab disease (Loria et al. 2008). The other draws upon a pair of molecules isolated from a strain of *Burkholderia rinojensis* (Bajsa-Hirschel et al. 2023; Owens et al. 2020), of which spliceostatin C is the principal active compound (Figure 2).

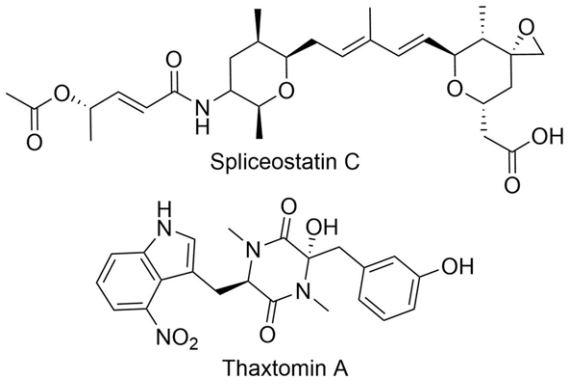

Figure 2. Microbially produced herbicidal molecules.

Spliceostatin C interferes with the production of mRNA through alternative splicing by binding to a pair of sites on the spliceosome previously known to be targeted by other phytotoxic compounds but representing a potential novel MOA in the commercial herbicide landscape (Bajsa-Hirschel et al. 2023). Many of the genes that plants regulate through alternative splicing are involved in responses to abiotic stresses and often do not overlap with those genes regulated through steady-state gene expression (Martín et al. 2021); it has been suggested that these differences occur consistently across many plant species, underlying the possibility of a wide spectrum of activity. In tests of spliceostatin C in planta, many weed species in the Amaranthaceae and Brassicaceae families show high levels of susceptibility, yet grasses and some other broadleaves appear tolerant. Therefore, the spectrum of activity may not be strictly determined by the molecular target site but may rather also be governed by other factors, such as structural and metabolic plant defense mechanisms or the physiochemical properties of the molecule itself. Approaching the spectrum of activity as the product of the complex interplay of these factors rather than as a static property of one molecule may allow for the spectrum to be altered through formulation additives and other means. This is an example of how understanding the scientific reasons underlying a potential commercial limitation for a bioherbicide (in the form of a narrower than desired spectrum of activity) can point the way toward potential technological solutions.

Efforts to develop a microbial bioherbicide may face additional technical and commercial obstacles beyond the perennial challenges in formulating for stability and optimized activity of sometimes complex fermentation products (Table 1). Reaching the desired titer of actives produced through the fermentation process can itself be a major technical challenge particular to microbial production systems. Secondary metabolites can be costly for microbes to assemble, so it is unsurprising to find that internal feedback mechanisms may be in place to restrict the levels of production, even under the optimum growth conditions (Ruiz-Villafán et al. 2022). Suboptimal levels of production may in turn dictate the need for downstream concentration steps to reach quantities of active compound(s) commensurate with expected field application rates; depending on the degree of concentration and the technology involved, the implications for cost of goods can be significant. For example, the amount of thaxtomin A that can be produced with the wild-type *S. acidiscabies* strain is limited to milligrams per liter, necessitating significant investments in concentration of the active molecule upon completion of the fermentation process. This in turn drove up the cost of production prohibitively. In spite of having been

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Table 1. Nature of some of the challenges encountered in developing a bioherbicide

Scientific	Technological	Commercial
Discovery	Production quantity	Cost per hectare
Chemical properties	Production cost	Storage
Activity	Concentration	Level of control
Culture	Production scaling	Application format
Formulation	Stabilization	Rate per hectare
		Existing programs

registered with the USEPA and possessing desirable attributes like pre- and postemergence activity, stability under a wide range of storage conditions, and an uncommon MOA (King et al. 2001), the low production titer of thaxtomin A in the fermentation in wild-type strains of *Streptomyces* has impeded the development of a marketable herbicide product. However, subsequent development of higher-yielding strains, for example, via mutagenesis techniques, has led to increases in thaxtomin A production by orders of magnitude, allowing for the development of a much more cost-effective production process. In this example, the largest barrier for development and commercialization of the bioherbicide was not in the inherent herbicidal properties of the active molecule, but rather in the technical process through which that molecule could be produced. Experiences such as these suggest that efforts to overcome the technical challenges in developing a microbial bioherbicide will generally be more resource-demanding and interdisciplinary than efforts to discover and characterize the activity of the herbicidal molecule itself.

Ultimately, the most significant obstacles to the development of a widely utilized bioherbicide may be commercial, hinging on the degree to which users are willing to modify their expectations. While all pesticides must meet certain thresholds of efficacy, cost, stability, and art-of-use criteria if they are to successfully penetrate the market, the expectations surrounding herbicides seem to be particularly elevated. For the past few decades, much of the herbicide market has been dominated by highly active broad-spectrum products sprayed over crops with engineered herbicide-resistance traits. As cases of resistance of weeds to one or another herbicide MOA have emerged, numerous traits have been "stacked" into the crops to enable them to tolerate application of herbicide products that mix multiple highly effective MOAs. In this milieu, growers routinely expect near 100% weed control, a standard of performance that is often not imposed on fungicides or insecticides. A newly introduced bioherbicide will likely be compared with such premixes of multiple active molecules, making it difficult to gain traction. While some segments of the market will find inherent value in the perceived environmental and human safety of stand-alone bioherbicides, this should not preclude herbicidal actives produced by microbes from being incorporated into premixes, paired with traits for tolerance in selected crops, or serving as a springboard for the synthesis of chemical analogues. In the long run, external factors like tightening government regulations, increasing public demand for perceived environmentally friendly growing practices, and cascading incidences of herbicide resistance may all contribute to gradually generating more favorable conditions for bioherbicides to be sought out and accepted by the market.

INBIOAR Strategy to Develop Plant Extracts to Kill Weeds

Plant–plant interactions offer a unique opportunity to study the biochemical interactions between plant species under field conditions. As plants are sessile organisms that cannot move from stressful situations, they have developed a great number of physical and chemical defenses to prevent the invasion of their space by insects, diseases, and other plants (Andersen et al. 2018; Callaway and Aschehoug 2000; Hierro and Callaway 2021; Jones et al. 2022; Kaur et al. 2022; Yactayo-Chang et al. 2020).

INBIOAR scientists have often observed a group of individual plants of one species, such as *Prosopis alba* for example, that grow together without plants of other species growing nearby or among them. Such intraspecies groupings somehow prevent the invasion of the same place by other plant species. "Monocultures" of the same plant species are also commonly found in large areas. How do such groupings occur?

The ability of a plant species to release a chemical that is toxic to other plant species competing for the same space may explain such vegetation groupings. Seeds from the species producing the chemical can germinate and grow, whereas other species will grow weakly or die. As a result, only one plant species will grow in an area. This phenomenon, known as allelopathy, refers to the effects of one plant species on another plant species through the release of chemical compounds called allelochemicals (Rice 1985). Different parts of a plant can deliver these compounds to the surrounding environment by leaching, root exudation, volatilization, organic matter decomposition, and other processes in both natural and agricultural systems.

Allelochemicals are key components of plant defenses against herbivory, microbial plant diseases, or other potentially competitive plants. Anticipating what plant species will be more successful through allelopathy is difficult, although observations of vegetation clustering, as described above, provide a rationale for collecting specific plant species for studies to search for novel phytotoxins (allelochemicals) that might be useful in weed management.

A Strategy to Survive Globally

Toothpickweed [*Ammi visnaga* (L.) Lam.] is a weed distributed worldwide. Originally, the species was studied for its medicinal properties. Its fruits have been described in pharmacopoeias as an antispasmodic, muscle relaxant, and vasodilator. Other uses in traditional medicine include treatment of mild angina symptoms, supportive treatment of mild obstruction of the respiratory tract in asthma or spastic bronchitis, and postoperative treatment of conditions associated with the presence of urinary stones (Gautam et al. 2007). This herb has also been used as a treatment for gastrointestinal cramps; as a diuretic; and as a treatment for vitiligo, diabetes, and kidney stones. Also, different extracts of this plant and their major constituents have antibacterial and antioxidant activities and prevent renal crystal deposition and cell damage caused by oxalate (Khalil et al. 2020). Thus, *A. visnaga* is rich in biologically active compounds.

This species grows preferentially under high sun exposure in clay soils, which are well drained and quickly desiccated on the surface, in the semiarid superior and subhumid bioclimatic zones (Sellami et al. 2013). In some regions, this plant has become an invasive weed of cultivated fields (Zandstra et al. 2004). *Ammi visnaga* plants were collected for extraction in a semiarid region of Argentina in an area of 900 by 30 m (Figure 3) completely covered by *A. visnaga*. This species is a successful invasive species. Native to the Mediterranean region of Europe, Asia, and North Africa, it is now found in Argentina, Brazil, Chile, Uruguay, North America, Southwest Asia, and some Atlantic islands.

Bioassay-guided isolation of phytotoxins from *A. visnaga* found that khelin and visnagin were the most active compounds

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Figure 3. *Ammi visnaga* at the beginning of the colonization process in a natural area of Argentina.

(Travaini et al. 2016). These metabolites were of previous interest to the pharmaceutical sector. The bioassay-guided isolation of the active ingredient(s) consisted of seed germination and plant growth bioassays in a laboratory. Postemergence herbicidal effects of *A. visnaga* extracts and the two isolated compounds were determined in greenhouse trials. Visnagin was the most active, with contact postemergence herbicidal activity on velvetleaf (*Abutilon theophrasti* Medik.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] at 2 kg ai ha^{-1}. Moreover, its effect at 4 kg ai ha^{-1} was comparable to the commercial bioherbicide pelargonic acid at the same rate. The initial formulation included only Tween® 20 (polisorbate 20) in water. Efforts are being made to improve the formulation of these active ingredients.

Khellin and visnagin may have more than one MOA. Their activity is not light dependent and involves effects on membrane stability, cell division, and cell viability in leaves and roots. These effects may not be related. Both compounds also reduce photosynthetic efficiency through indirect effects and induce oxidative damage under high light intensity.

Additionally, analogues of khellin and visnagin were synthesized, and their herbicidal activities were examined (Cantrell et al. 2023). Acetate analogues of khellin and visnagin had more activity on lesser duckweed (*Lemna paucicostata* Hegelm.; syn. *Lemna aequinoctalis* Welw.) than visnagin, and the *O*-demethyl butylated visnagin analogue was the most active compound with an IC_{50} of 47.2 µM. In additional herbicide bioassays, visnagin and the *O*-demethyl butylated visnagin analogue were the most active compounds and reduced the germination and early growth of lettuce (*Lactuca sativa* L.) and ryegrass species (*Lolium* spp.), translocated from the solution through the plant shoots of white mustard (*Sinapis alba* L.) and corn, and reduced the canopy cover of foxtail millet [*Setaria italica* (L.) P. Beauv.] and *S. alba* after postemergence application.

Progressive Effect of a Plant Extract

Most of the plant-based bioherbicides produce burning or contact effects. A weakness of "burndown/contact" herbicides, whether bioherbicides or synthetic, is that treated plants tend to regrow from meristems that did not come in contact with the herbicide. Therefore, INBOAR's goal was to identify a phytotoxin with a systemic MOA.

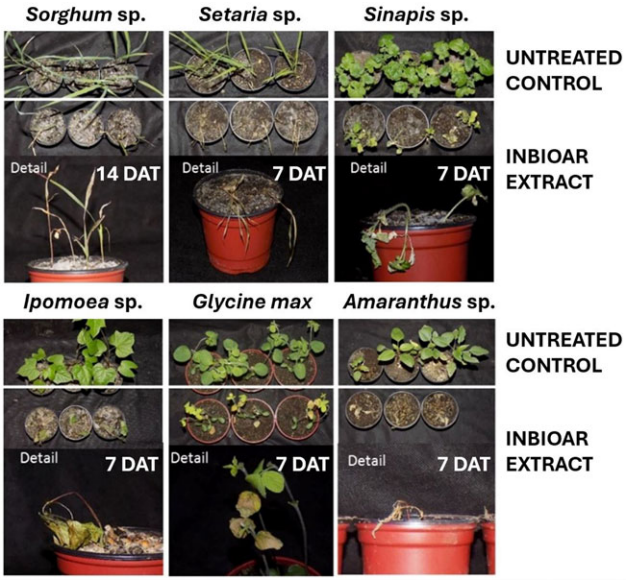

Figure 4. A plant extract that kills weeds slowly and systemically when applied postemergence. Effects are 10 d after treatment.

a plant extract with a non-immediate and more systemic herbicidal effect was found by screening the flora in semiarid regions. This aqueous extract kills the weeds progressively after postemergence application (Figure 4). Within 10 d of plants being sprayed with an aqueous solution of the extract (20 mg ml^{-1}), high control (80% to 100%) of different species, with activity on both dicotyledonous and monocotyledonous species, was observed. *Sorghum* sp., *S. italica*, *Sinapis* sp., morningglory (*Ipomoea* sp.), Palmer amaranth (*Amaranthus palmeri* S. Watson), and soybean [*Glycine max* (L.) Merr.] were among the most sensitive species, while lettuce, corn, oat (*Avena fatua* L.), clover (*Trifolium* sp.), and *Lolium* sp. were affected moderately or not at all.

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The herbicidal efficacy was tested at different doses, and some weeds—green foxtail [*Setaria viridis* (L.) P. Beauv.], redroot pigweed (*Amaranthus retroflexus* L.), and wild poinsettia (*Euphorbia heterophylla* L.)—were still controlled (80% to 100%) at 15 and 7.5 kg ha^{-1}. The effect was improved with the use of different adjuvants. The active ingredient(s) of this extract have not been identified yet. However, the efficacy on weeds is consistent, and the purification of and identification of the active ingredient is in progress.

What Has Been Learned?

The flora is a source of natural active ingredients. The evolutionary forces involved in different plant species fighting for resources in nature has driven the development of novel secondary metabolic pathways, sometimes resulting in the production of potent phytotoxic allelochemicals that could be useful for crop protection. Indeed, the triketone herbicides were the result of discovery of the phytotoxicity of the triketone allelochemical leptospermone (Knudsen et al. 2000; Lee et al. 1997).

The crop protection industry is waiting for answers from the scientific community. Both Bayer CropScience (Testing4Ag) and BASF (free in vivo testing) have initiated programs to test bioactive compounds discovered by academia and other institutions as potential leads for new pest management tools. It is incumbent on the scientific community to contribute to these industry efforts to discover novel active ingredients for pest control. But the future is not exclusively dependent on conventional, synthetic chemical tools. Novel and safer agricultural systems are needed, where environmentally friendly products can be incorporated as management tools to produce crops and vegetables.

MicroMGx Strategy to Accelerate Phytotoxic Natural Product Discovery through Metabologenomics

Metabologenomics is a powerful tool driving innovation in applied science, particularly in the field of natural product discovery. By seamlessly integrating genome sequencing, genome mining, and state-of-the-art metabolomics techniques like mass spectrometry and nuclear magnetic resonance, this methodology empowers researchers to uncover novel bioactive compounds with real-world applications (Doroghazi et al. 2014; Goering et al. 2016). At its core, metabologenomics offers a practical approach to navigating the complex landscape of microbial secondary metabolite biodiversity. Through systematic analysis of BGCs and metabolomics data, researchers can efficiently identify promising candidates for further investigation (Yan et al. 2018). This targeted approach allows for the rapid identification and characterization of natural products with potential pharmaceutical, agricultural, or industrial uses. One of the key strengths of metabologenomics lies in its ability to prioritize the discovery of new secondary metabolites. By leveraging advanced computational pipelines and bioinformatics tools, researchers can pinpoint BGCs associated with previously unexplored chemical scaffolds. This targeted screening approach significantly accelerates the pace of natural product discovery, facilitating the development of novel therapeutics, agrochemicals, and biotechnological applications. Furthermore, metabologenomics enables researchers to tailor their searches for specific classes of natural products based on predefined criteria. Whether targeting polyketides, non-ribosomal peptides, phosphonates, or other specialized metabolites, this methodology provides a customizable framework for targeted screening and discovery. By harnessing the power of genomics and metabolomics, MicroMGx can unlock the full potential of microbial diversity and harness nature's vast biochemical repertoire for practical applications.

Exploring the Potential of Phosphonate Natural Products in Agriculture

Phosphonate natural products represent a promising group of compounds for biocide discovery due to their potent bioactivities. Despite their significance (Ju et al. 2014; Yu et al. 2013), this class of compounds remains relatively underexplored compared with other classes of natural products. The evolutionary mechanism underlying phosphonate inhibition highlights the strategy of molecular mimicry through the structural resemblance to phosphate esters and carboxylic acid metabolites (Figure 5).

The chemically inert nature of the stable phosphorus–carbon bond in phosphonates confers their remarkable specificity and potency as enzyme inhibitors. By competing with their structural analogues for enzyme binding, phosphonates disrupt normal catalytic functions, offering a targeted approach to enzyme inhibition. Given the ubiquitous roles of phosphate esters and carboxylic acids in biological processes, the spectrum of potential cellular targets for phosphonate inhibitors is vast. In practical applications, phosphonates have demonstrated their efficacy as active ingredients in widely used herbicides such as glyphosate and glufosinate, showcasing their practical utility in agriculture.

Plant-associated Enterobacteria as Sources for Herbicidal Molecules

The escalating problem of pesticide resistance in agriculture poses a significant threat, leading to substantial losses in global crop yields, estimated at up to 50% (Gould et al. 2018). Efficient and targeted discovery efforts rely heavily on access to diverse and reliable sources of new compounds. Host-associated enterobacteria emerge as promising candidates, offering a rich reservoir of bioactive natural products (Adnani et al. 2017; Pidot et al. 2014). These microorganisms boast attributes conducive to laboratory cultivation and genetic manipulation, leveraging well-established techniques developed for their close relative, *Escherichia coli*. Utilizing straightforward genetic engineering methods, such as promoter exchange (depicted in Figure 6, right), enables the native expression of BGCs. This approach simplifies the process of accessing and studying the bioactive potential of these compounds, circumventing the need for complex heterologous expression systems that frequently encounter inefficiencies and failures. Given the urgent demand for new phytotoxic agents and the well-established bioactivity of phosphonates, adopting a metabologenomics approach centered on plant-associated enterobacteria emerges as a viable strategy for pinpointing herbicides with potentially new MOAs (Figure 6).

The application of metabologenomics to explore the bioactive potential of phosphonates from plant-associated enterobacteria represents a promising approach to addressing the urgent need for novel herbicides. This applied discovery approach holds promise for revolutionizing agricultural practices and mitigating the impact of pesticide resistance on global crop production.

Discovery of Pantaphos from a Plant-associated Enterobacterial Pathogen

Pantaphos, a novel phosphonate compound originating from strains of *Pantoea ananatis*, a common plant pathogen, was recently discovered. Pantaphos has remarkable phytotoxic properties, including inducing the characteristic lesions associated with onion center rot (Polidore et al. 2021). Our investigations revealed

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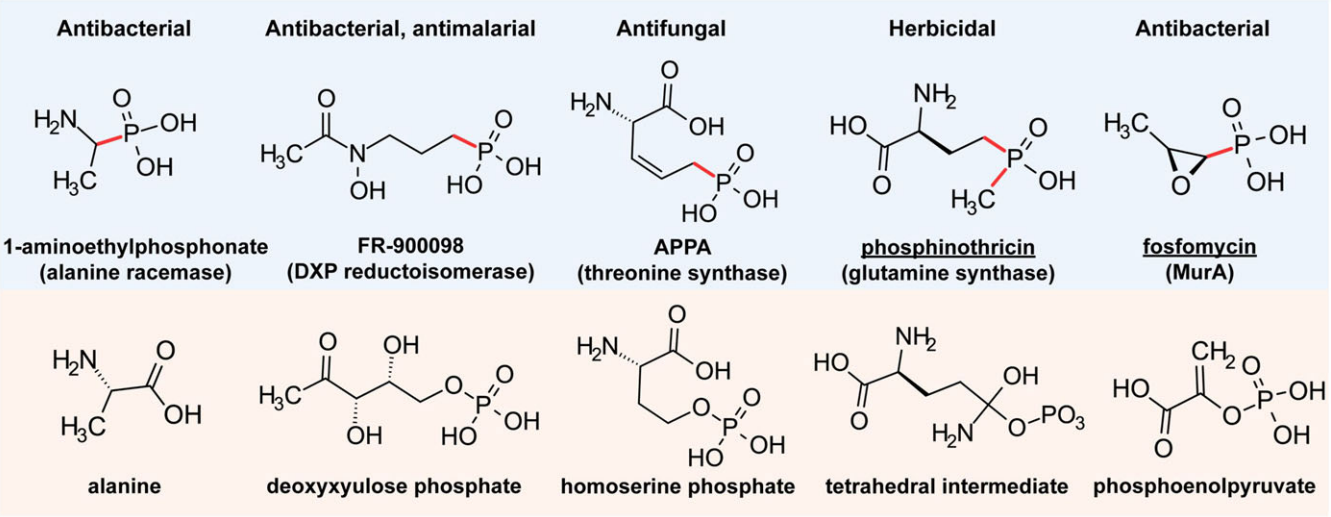

Figure 5. Structure and bioactivity of naturally occurring phosphonates and their molecular mimics. Bioactive phosphonates with their respective enzyme targets are shown with a light blue background. The enzyme's native substrates are shown in a light red background. Phosphorus-carbon bonds are shown as bold red lines on the molecule, and commercially available compounds are underlined. Fosfomycin, clinically sold as Monurol®, is used to treat difficult urinary tract infections, and phosphinothricin (also known as glufosinate) is the active ingredient for the broad-spectrum commercial herbicide sold under several trade names.

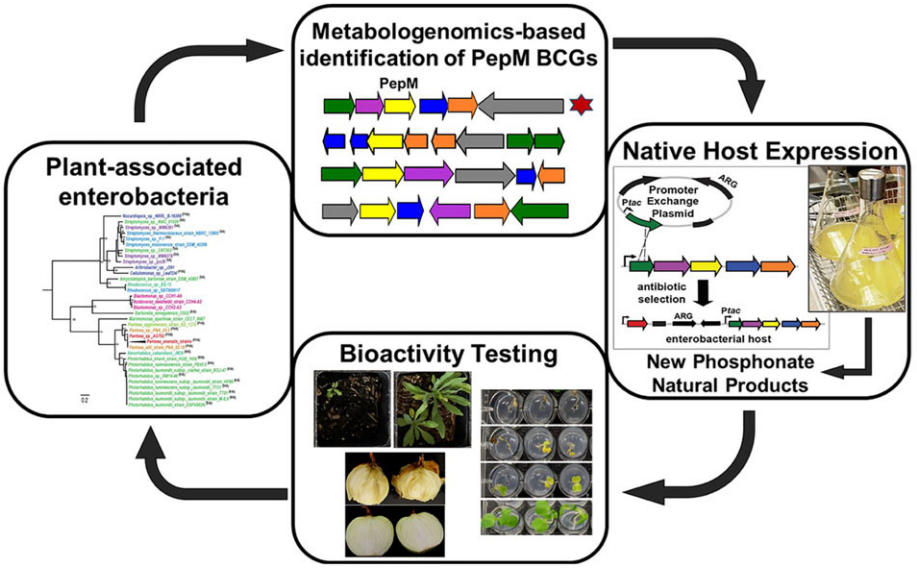

Figure 6. Discovery of novel herbicides from plant-associated enterobacteria (left). Novel phosphonate biosynthetic gene clusters (PepM BGCs) are identified from plant-associated enterobacteria through the metabologenomics platform (top, center). Native host expression of the PepM BGC is achieved by introduction of an inducible promoter, Ptac, through a simple promoter exchange method via homologous recombination (right). A bacterial recombinant with the exchanged promoter is then selected for using the antibiotic resistance gene (ARG). Spent media or purified compounds are then used for herbicide bioassays (bottom, center).

potency that is comparable to widely used herbicides like glyphosate and phosphinothricin. With *P. ananatis* showing a wide host range and the prevalence of its BGC among pathogenic strains, the potential bioactivity of pantaphos across various plant species is noteworthy. To assess its efficacy, an efficient, scalable production method for pantaphos production was devised using the native host. After purifying the compound from spent media, it was formulated for greenhouse testing. The resulting product, MGX-1001, demonstrated significant growth inhibition in numerous crops and weed species, particularly broadleaf weeds (Figure 7).

Intriguingly, MGX-1001 had minimal impact on key food crops like corn, oat, wheat (*Triticum* spp.), and barley (*Hordeum vulgare* L.), suggesting practical applicability in agricultural fields. Moreover, our findings indicate that MGX-1001 effectively suppresses the growth of herbicide-resistant weeds, hinting at a potential new MOA. While further investigations are warranted, initial experiments suggest a unique mechanism of action targeting broadleaf weeds specifically. Notably, our ongoing research includes the identification of a resistance allele that can be expressed in plants, offering insights into potential genetic modifications in plants for enhanced herbicide tolerance.

With its promising properties, including a suspected novel MOA and compatibility with broadleaf weed control, pantaphos emerges as a next-generation herbicide with significant agricultural implications. Our focus now shifts toward refining formulation techniques and advancing commercialization efforts, leveraging pantaphos's natural origin and its anticipated impact on

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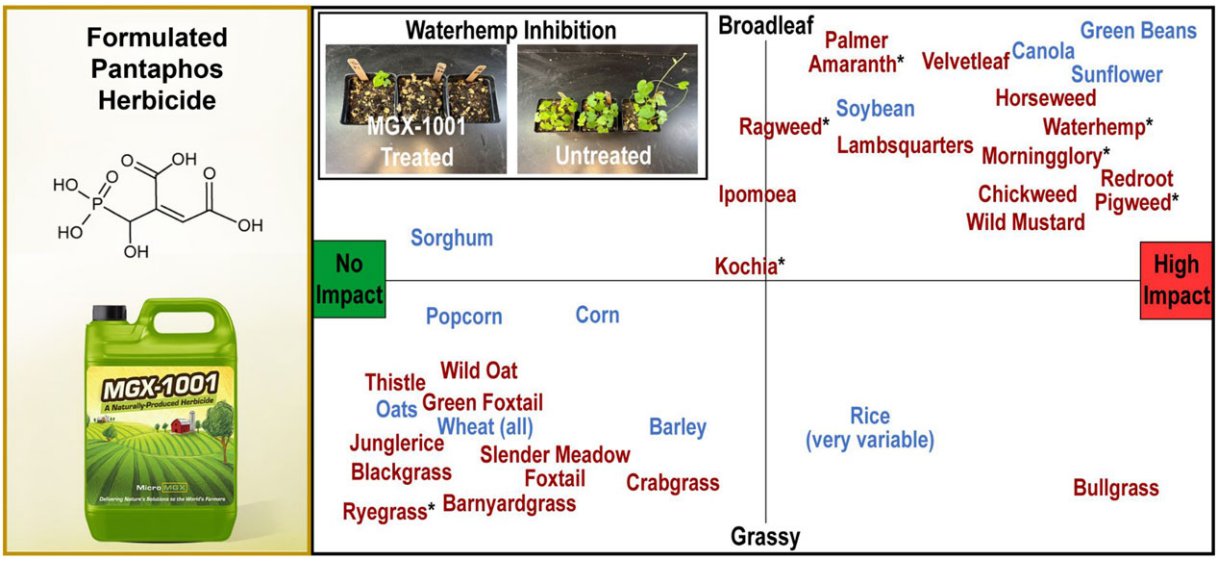

Figure 7. Growth impact assessment among common crop and weed species treated with formulated pantaphos. Data were compiled from five independent greenhouse trials with crop species in blue and weed species in red. The asterisks denote weed species with known glyphosate resistance. Horizontal axis is measured impact assessment based on overall growth yield compared with an untreated sample, and vertical axis distance is arbitrary. The growth phenotype of waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] after treatment with pantaphos is shown in the upper left inset. Rice (*Oryza sativa* L.) showed highly variable growth impact that was found to be dependent on the adjuvant used in the pantaphos formulation.

weed management. Also, the exploration of phosphonate natural products presents opportunities for scientists seeking innovative solutions in agriculture and beyond (Manghi et al. 2021).

Biochemical, Molecular, and Computational Approaches

Herbicide discovery has historically relied on biological activity–driven approaches, and all the main agrochemical companies still base a large part of their discovery programs on the combination of chemical synthesis of diverse chemistry and whole-plant screens. Using that model, the major chemical companies screen between 10,000 and 60,000 new molecules per year. A series of assays are usually used to first identify active molecules and provide preliminary information such as selectivity profiles, pre- versus postemergence activity, and robust symptomology information to flag compounds with potential new MOAs. Lead compounds arising from these assays are then tested for activity on herbicide-resistant weeds and biochemical assays to assess their MOAs. The costs associated with bringing a new active ingredient to markets between 2014 and 2019 amounted to US$302 million, including US $127 million in discovery research, US$133 million in research development and toxicological studies, and US$42 million for registration (AgbioInvestor 2024). The average time between discovery and registration exceeds 12 yr.

As there has been a dearth of new MOAs, several start-up companies have designed novel platforms to speed up the discovery process.

Moa Technology is a UK-based company that aims to discover the undiscovered by harnessing the principles of natural selection on miniature plants in concert with rapid phenotyping to find new MOAs, BioHeuris is an Argentinian company that developed two technology platforms to develop next-generation herbicide-resistant crops using protein engineering and gene editing, and U.S.-based Oerth Bio capitalizes on the natural plant protein recycling system as a tool for crop protection and plant health. On the other hand, the Israeli company Projini is developing new pesticides that interfere with protein–protein interactions. Enko, a company based in the United States, is making strides in using combinatorial chemistry and DNA-encoded libraries, artificial intelligence (AI), and machine learning to explore vast new chemical spaces for herbicide discovery. Finally, the Israeli company Agrematch is a data-driven small molecules discovery company based on a powerful AI machine/deep learning compound platform.

GALAXY, a High-Content Imaging Platform Enabling Novel Herbicide Discovery

Moa Technology (www.moa-technology.com) is a start-up company created in 2017 by a group of researchers at the University of Oxford (UK) to tackle the problem of weed resistance in crop production. Two main strategies have traditionally been deployed by the industry to discover herbicides with novel MOAs: phenotypic and in vitro targeted approaches (Hachisu 2021). Moa Technology has developed an innovative and systematic large-scale in planta herbicide discovery platform that enables high-throughput discovery of novel classes of MOAs with unique and potentially powerful herbicide activity an order of magnitude faster than traditional methods.

More than three-quarters of a million synthetic and natural product compounds have been screened through GALAXY. Glasshouse performance in Moa Technology facilities for compounds displaying novelty has been extensively profiled and led to the identification of more than 60 moaNOVEL chemical areas (Figure 8). moaNOVEL areas are chemical areas empirically discovered to likely act with a new MOA by Moa Technology's proprietary miniaturized plant-led high-content phenotyping assay used to screen an enormous diversity of natural and synthetic chemistries on microscopic whole plants and rapidly identify an abundance of new, never commercialized herbicide MOAs at unprecedented scale, speed, and cost.

GALAXY answers the question "Is it a herbicide, and does it work in a new way?" by comparing the extensively profiled dose-

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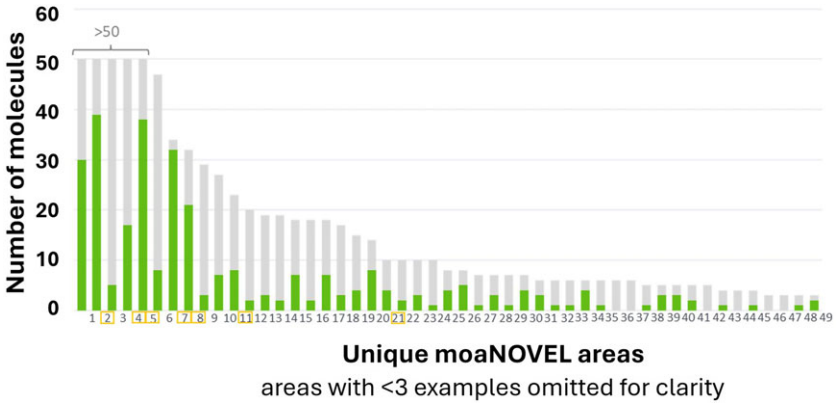

Figure 8. Graphical representation of moaNOVEL families identified by the GALAXY platform. Unique moaNOVEL families with more than 50 representative molecules are denoted with a bracket (>50). The number of each moaNOVEL molecule active in weeds is highlighted in green. moaNOVEL families with glasshouse activities are highlighted in yellow.

dependent plant symptoms associated with tested molecules. The GALAXY platform discriminates between hundreds of molecules belonging to known MOA classes and novel molecules eliciting the unique, never-observed plant symptomologies that are specifically associated with the urgently needed moaNOVEL areas. Using high-content imaging, GALAXY characterizes nearly 100 individual parameters for each plant exposed in each well to unique compounds sourced for their diversity from vast compound libraries. A proprietary digital segmentation and categorization process for each plant then ensures the classification of every molecule in known or moaNOVEL categories, paving the way for the elucidation of their MOAs.

Once a novel molecule has been identified by GALAXY, the next step is to understand the MOA, the molecular target, and the pathways modulated by each molecule. To ensure a successful outcome, a combined constellation of powerful complementary screens is simultaneously mobilized. In this way, the new MOA, molecular target or pathway can be qualified, thus answering how the herbicide works and evaluating its probable safety and potential commercial opportunities. Triggered early to de-risk the next-generation herbicide discovery effort, the process delivers results in months rather than years, accelerating predictions of safety and optimization into farm-ready herbicides.

Moa Technology is advancing a growing pipeline of novel synthetic compounds and bioherbicides toward field trials at pace (Figure 9). Leveraging the suitability of GALAXY for testing minute amounts of compounds on whole plants, Moa Technology is shortening even further the path to market by screening natural compound libraries. Natural product herbicides have the potential to deliver commercial success more rapidly than synthetic counterparts, including natural products acting via known MOAs. Natural extract libraries derived from plants, fungi, bacteria, or marine organisms have already been successfully profiled, and selected candidates are advancing in further glasshouse testing. The speed and power of the approach taken for novel synthetic and natural product compounds means that international field trials in both the Northern and Southern Hemispheres are being initiated in 2024 with multiple lead molecules with new MOAs.

Heurik™ and Swap™—Two Integrated Platforms to Optimize Crop Genes for Herbicide Resistance

CRISPR gene editing is a relatively new technique that enables the introduction of precise mutations in plants (and other organisms) that can include one or several nucleotide changes (Jiang et al. 2013). This new tool is opening new opportunities to create crops with novel traits (Scheben et al. 2017). Moreover, in many countries, gene-edited crops derived from CRISPR technology are not considered genetically modified organisms (GMOs) and can be commercialized as conventional crops (Duarte Sagawa et al. 2024; Sprink et al. 2022). This avoids the expensive and time-consuming regulatory process associated with GMO crops and circumvents current public concerns over GMO foods (Ryan et al. 2024).

In plants, mechanisms that provide herbicide resistance can be classified as target site or non−target site, depending on whether the resistance is related to the protein that is inhibited by the herbicide or caused by other factors (for a review, see Gaines et al. [2020]). Both approaches have been used to develop herbicide-resistance traits either by engineering genetically modified plants that express genes from other organisms (transgenic or GMO) or by traditional mutagenesis of native genes in crops (Duke 2015; Green 2014). Nonetheless, traditional mutagenesis is a slow process that introduces random mutations that are limited in type. On the other hand, bringing a GMO to the market takes more than 16 yr and costs more than US$100 million (AgbioInvestor 2024). BioHeuris has developed two technology platforms that integrate protein engineering (Heurik™) and gene editing (Swap™) to develop next-generation herbicide-resistant crops in under 6 yr and at a cost 50 times lower.

Protein engineering is based on the modification of protein sequences through substitution, deletion, or insertion of nucleotides in the encoding gene. This process can be used to identify mutations in plant genes that provide target-site or non−target site resistance without affecting the fitness of the plant. BioHeuris developed Heurik™, a high-throughput microbial platform, to discover and measure the level of herbicide resistance in plant genes carrying different mutations (Figure 10). In a few weeks, this platform can mimic experiments that would take hundreds of hectares and years of field trials if done with traditional mutagenesis. Heurik™ uses directed evolution and rational design as the two main strategies for protein engineering. The directed evolution strategy involves generating a library of millions of gene variants and screening for herbicide resistance in engineered microbes. The rational design strategy uses computational models to infer which amino acid changes could provide the desired resistance while retaining the protein activity or function.

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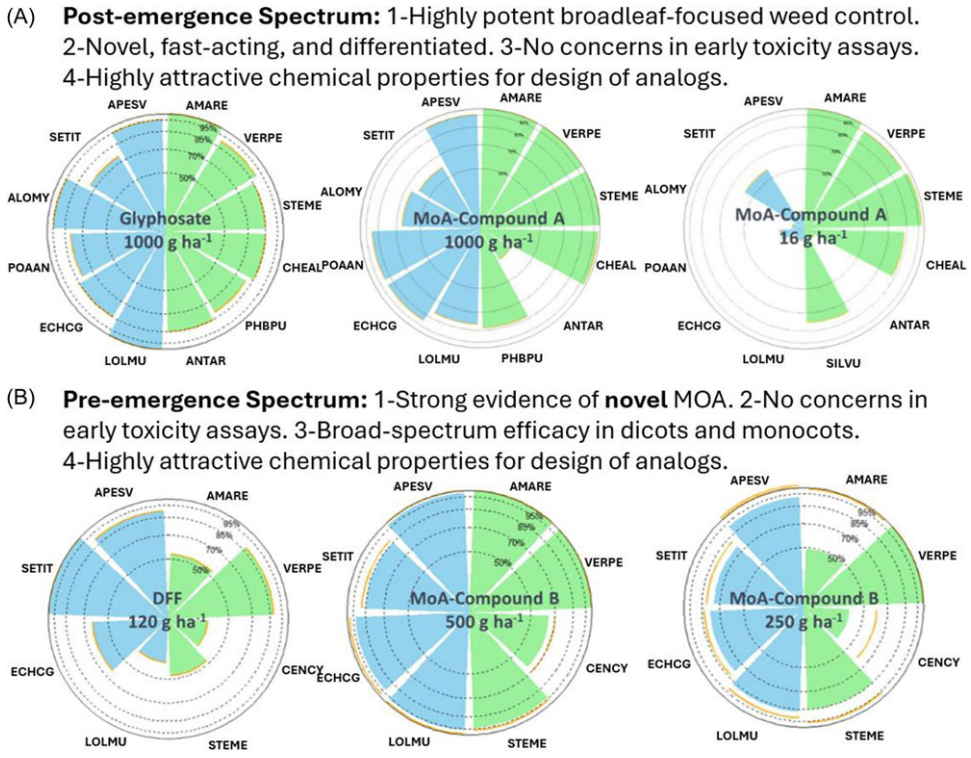

Figure 9. Weed spectrum evaluation of two examples of molecules being shortlisted for field trials in 2024: (A) molecule with broadleaf spectrum and largely postemergence properties and (B) molecule with largely preemergence properties and broad spectrum. DFF, diflufenican; ALOMY, blackgrass (*Alopecurus myosuroides* Huds.); AMARE, redroot pigweed (*Amaranthus retroflexus* L.); ANTAR, chamomile [*Anthemis arvensis* (Wallr.) DC.]; APESV, common windgrass [*Apera spica-venti* (L.) P. Beauv.]; CENCY, cornflower (*Centaurea cyanus* L.); CHEAL, lambsquarters (*Chenopodium album* L.); ECHCG, barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] LOLMU, Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot; PHBPU, tall morningglory (*Ipomoea purpurea* (L.) Roth); POAAN, annual bluegrass (*Poa annua* L.); SETIT, foxtail millet [*Setaria italica* (L.) P. Beauv.]; STEME, common chickweed [*Stellaria media* (L.) Vill.]; VERPE, birdeye speedwell (*Veronica persica* Poir.). Blue indicates grass weeds; green indicates broadleaf weeds.

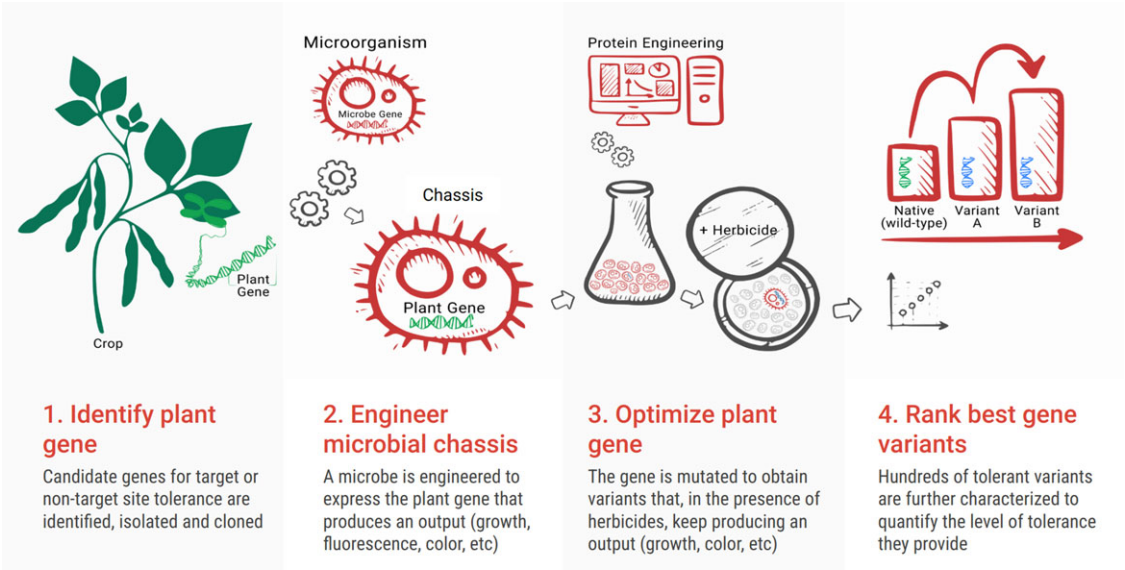

Figure 10. Heurik™ trait-discovery workflow developed to identify candidate mutations in plant genes that provide herbicide resistance.

The identified mutations are then introduced in crops by BioHeuris using Swap™, a proprietary gene editing platform. Swap™ uses different versions of CRISPR enzymes to introduce short deletions or nucleotide substitutions (Figure 11). This platform combines gene editing with in vitro tissue culture of elite lines and speed breeding to generate edited crops in 1 yr without leaving DNA from other species in plant genomes (Ghosh et al. 2018).

A different approach to identifying mutations by protein engineering could be to use mutations previously discovered in herbicide-resistant weeds. However, if the herbicide is new, this source of resistance might not be present in natural populations.

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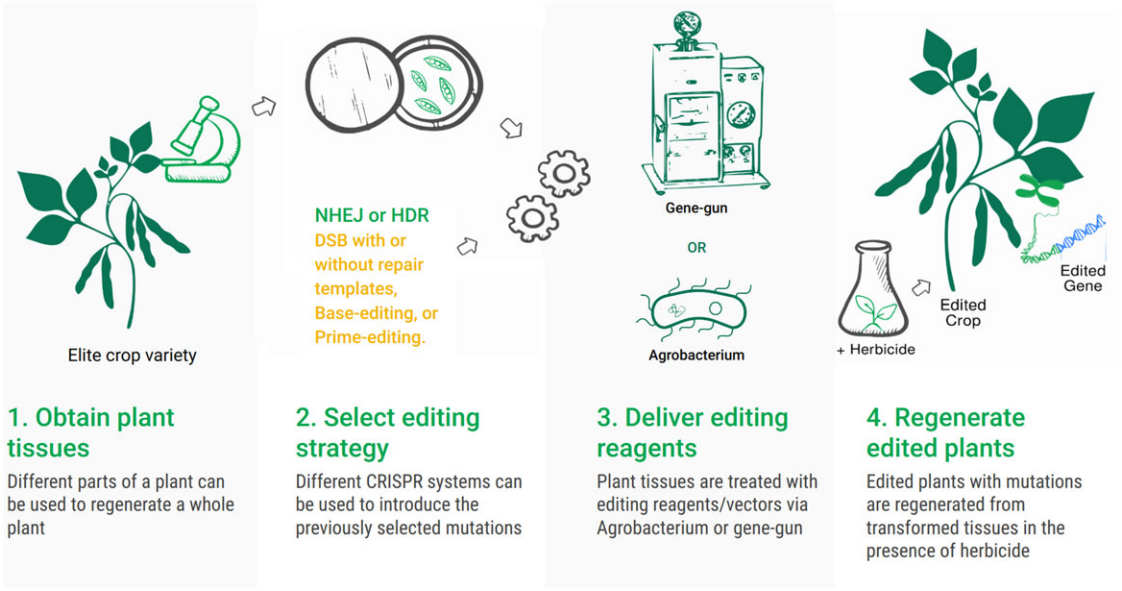

Figure 11. Swap™ gene editing workflow allows the creation of mutations in plant genomes to efficiently obtain herbicide-resistant elite varieties. NHEJ, non-homologous end joining; HDR, homology-directed repair; DSB, double-strand break.

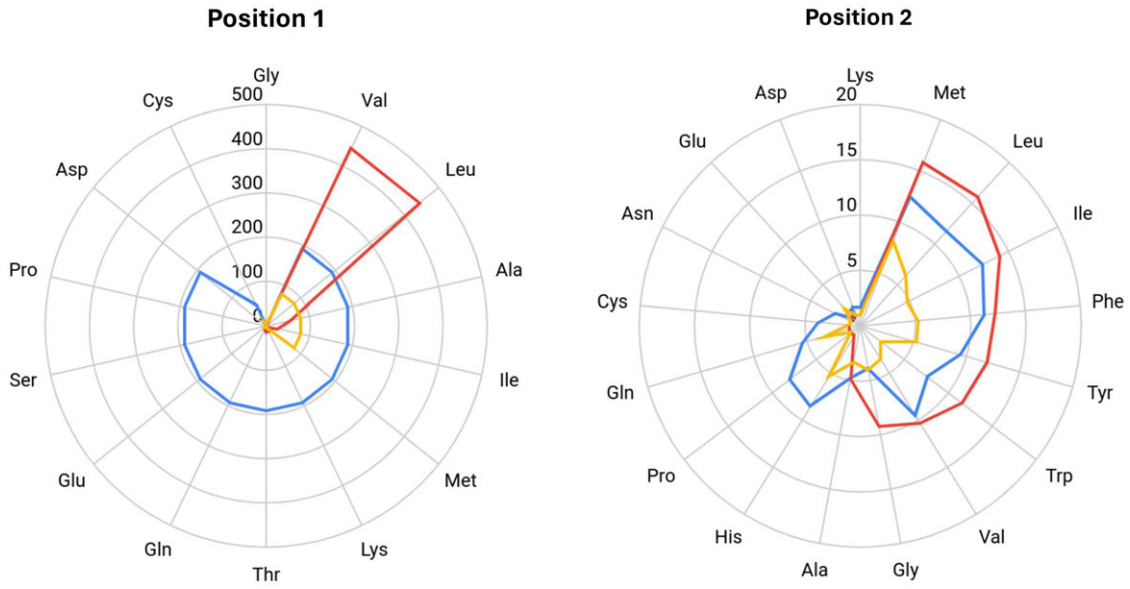

Figure 12. Herbicide resistance provided by different amino acid changes in two conserved positions of an orthologous target-site enzyme from soybean (red), sorghum (blue), and cotton (yellow). Changes are indicated with the three-letter code for each amino acid. Values correspond to the resistant/susceptible (R/S) ratio, calculated as GI_{50} of mutant variant by the GI_{50} of the wild-type variant.

Also, empirical evidence suggests that replicating known mutations in target-site enzymes across different plant species might not provide the expected herbicide resistance in all of them. This was shown by introducing equivalent single amino acid changes in target-site enzymes from different crops and measuring not only different levels of resistance but whether some mutations had a positive effect only in certain species. The work was done by scientists from BioHeuris who replaced a microbial gene with the plant version of a target-site enzyme from soybean, sorghum [*Sorghum bicolor* (L.) Moench], and cotton (*Gossypium hirsutum* L.) (60% to 70% identity). They then introduced all possible (19) changes in conserved amino acid positions and measured growth inhibition of the microbial "chassis" species at increasing amounts of herbicides (Figure 12).

At least in some cases, predicting and identifying mutations that can provide resistance might be crop specific, calling for the development of high-throughput cost-efficient discovery methods. By combining the described synthetic biology platforms, BioHeuris developed herbicide-resistant sorghum and rice elite varieties and validated them in field trials in only 3 yr. Several countries, including Argentina, Brazil, the United States, Chile, and Colombia, already confirmed that these plants are not GMO.

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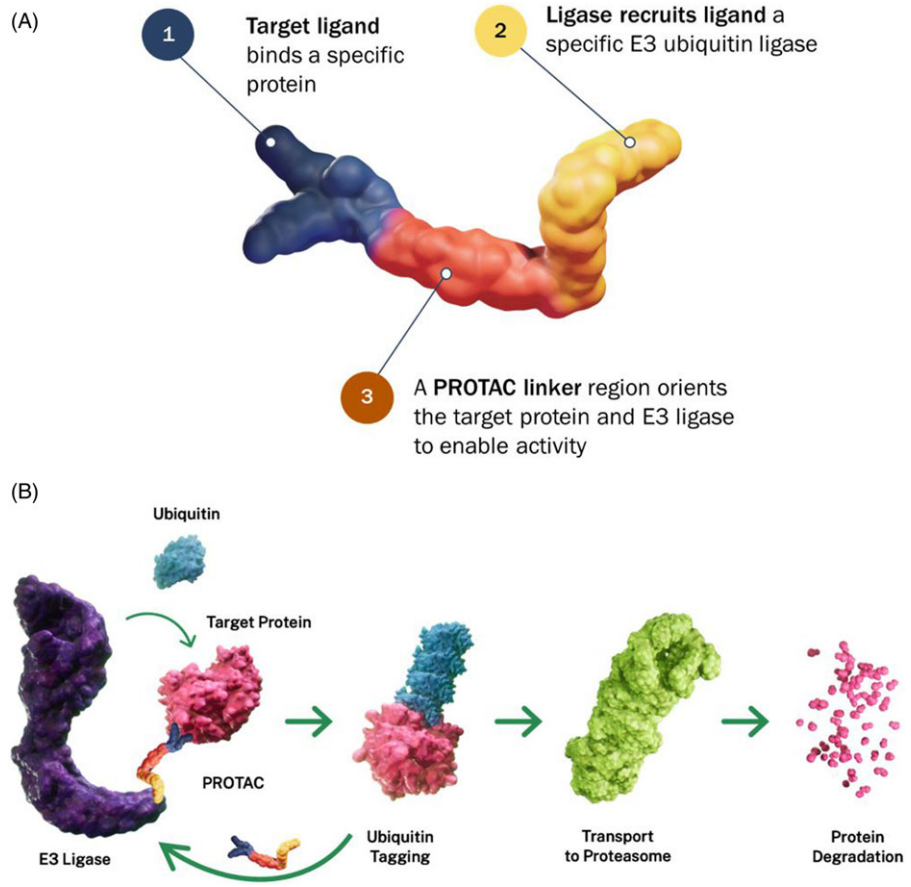

Figure 13. (A) Illustration of a proteolysis-targeting chimera (PROTAC) with (1) its target ligand binding the target protein of interest, (2) its ligase-specific ligand to recruit a specific E3 ubiquitin ligase, and (3) its linker that covalently attaches those two ligands. (B) Illustration of the PROTAC mechanism of action (MOA): the PROTAC recruits a specific E3 ligase and a target protein to form a ternary complex, allowing several molecules of ubiquitin to be transferred onto the surface of the target protein. The ubiquitin-tagged protein is then transported to the proteasome for degradation. Figure reproduced with permission from Oerth Bio, LLC.

Pre-commercial trials are underway, and BioHeuris expects that it will only take 6 yr to bring these traits to farmers with a cost 50 times lower than a GMO event.

ATTUNE™—A Novel Platform Harnessing Targeted Protein Degradation for Agricultural Use

Novel, non−enzyme inhibition approaches are needed for the expansion of new herbicide MOAs. Inspiration can be drawn from the pharmaceutical industry, especially in the field of targeted protein degradation (TPD). TPD has expanded rapidly over the past two decades, with more than 40 companies exploring this technology. PROTACs, or proteolysis-targeting chimeras, represent the most advanced chemistry now progressing to commercialization (Békés et al. 2022). Similar to the adoption of CRISPR technology, PROTACs are poised for use in agriculture with an emphasis on creating safe, precise, and environmentally friendly chemistries.

PROTACs emerged in the early 2000s as a groundbreaking concept pioneered by Craig Crews and colleagues at Yale University (Bondeson et al. 2015; Zengerle et al. 2015). PROTACs are biorationally designed, modular compounds that target specific proteins within cells for degradation by harnessing the endogenous cellular machinery responsible for protein degradation (proteasome). These synthetic molecules consist of three main components: (1) a ligand binding to the protein target of interest (POI), (2) a ligand binding to an E3 ubiquitin ligase enzyme, and (3) a linker that covalently binds those two ligands (Figure 13A).

Upon binding to both the E3 ligase and target protein, a PROTAC-mediated ternary complex forms and facilitates the transfer of ubiquitin onto the surface of the target protein by the E3 ligase. This ubiquitination flags the protein for degradation and recycling by the ubiquitin proteasome system (Figure 13B). Once the ternary complex dissociates, the PROTAC molecule can be released to engage in further rounds of degradation, which is referred to as the "catalytic mechanism." This groundbreaking catalytic effect enables much lower amounts of compound to achieve the desired efficacy (Bondeson et al. 2015). Additionally, PROTACs can typically achieve more sustained and complete efficacy compared with classical inhibitors, as they remove the POI from the cell entirely rather than merely blocking its function (Burslem et al. 2018). Importantly, PROTACs do not require a traditional active site to initiate degradation, which opens up the protein landscape to targets previously considered "undruggable" (Liu et al. 2021).

While the vast majority of the PROTAC development is strictly focused on human health, Oerth Bio is the lone pioneer in developing PROTACs for agricultural use. Oerth Bio was founded in 2019 as a joint venture between Bayer CropScience and Arvinas. The company was created to develop the next wave of novel crop protection tools for agriculture (Speake et al. 2023). Since its

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inception, Oerth Bio has developed a proprietary platform called ATTUNE™, which is the first agricultural PROTAC discovery engine. The backbone of ATTUNE™ is Oerth Bio's E3 ligase discovery and ligand identification pipeline. As E3 ligases are essential for enabling PROTAC activity, Oerth Bio focuses on identifying kingdom- and phylum-specific E3s to dial-in applications specific to crop protection. Once a suitable E3 ligase is identified, two key steps are critical for making it PROTAC ready: (1) confirmation of its degradative abilities with one or several POIs using a dedicated biological assay and (2) identification of a small molecule ligand to bind and recruit that E3 ligase. Once those two key milestones are achieved, the Oerth team pairs the ligase with a compatible protein target for actual PROTAC development. Unlike traditional "spray and pray" or phenotype-driven discovery, PROTACs are rationally designed to ensure the specific degradation of the POI. This design encompasses a unique set of computational and biological tools in combination with a team of synthetic chemists. Once protein degradation is achieved, the PROTAC efficacy can be significantly enhanced through rapid cycles of design, build, and test to afford a high enough level of degradation potency.

With more than 1,000 E3 ligases in plants, there is a tremendous opportunity to develop novel herbicides (Mazzucotelli et al. 2006). PROTACs offer three main advantages over traditional MOAs: (1) improved safety and specificity through tunability, (2) lower dosing with sustained efficacy, and (3) protein target expansion.

The first key feature of PROTACs for herbicide discovery is related to their overall tunability. Because their chemistry is modular, each of the three components of a PROTAC can be individually tailored to dial-in unique properties, including safety and specificity. The first level of tunability is linked with the actual E3 ligase selection. For instance, using a solely plant-specific E3 ligase significantly decreases the likelihood of off-target effects. The second level of tunability is within the E3 ligase and the protein target chemistry. Because active site orthosteric ligands are not strictly necessary for PROTAC activity, a larger chemical space can be utilized for PROTAC design, especially when it comes to ligands with a fairly wide range of binding affinities. Ligands that bind at allosteric sites and other nontraditional surface pockets further increase this chemical flexibility, making the different E3 binder–linker–POI binder combinations nearly infinite. Finally, the PROTAC linker can be further fine-tuned for the safety, selectivity, and delivery of these molecules. The linker is not only essential for the formation of the ternary complex and protein degradation, but also allows for tuning the overall physical chemistry properties of the PROTAC (Atilaw et al. 2021). Collectively, these multiple layers of tunability provide a path for a more precise and safe-by-design herbicide.

The second key feature concerns the fact that PROTAC-based herbicides have the potential to reduce the environmental impact for farmers by allowing for lower use rates compared with traditional chemistries. This is a direct consequence of the catalytic mechanism of PROTACs, which enables the degradation of multiple copies of the POI by a single PROTAC molecule (as opposed to traditional small molecule inhibitors possessing a 1:1 stoichiometry with the POI). This amplification effect has the potential to dramatically lower the use rate by 10 to 100 times. Additionally, the catalytic mechanism could provide longer-lasting effects, requiring fewer field applications. Combined with the tunability of these molecules, there is an additional opportunity to replace environmentally unfavorable chemistries.

The last key feature of PROTAC-based herbicides is their promising ability to expand the protein target landscape but also to rescue old herbicide molecular targets with known, effective herbicide inhibitors. Commercial herbicides target a very limited range of weed proteins, characterized by well-defined binding sites. Additionally, many research targets have never been commercialized due to selectivity, potency, and safety concerns. Furthermore, the overuse of existing chemistries has led to evolution of significant resistance in the field. PROTACs offer a variety of avenues to expand and overcome all these issues. First, designing PROTACs against existing commercial protein targets can allow for lower use rates compared with current active ingredients due to the catalytic effect, which can improve safety profiles as well as limit the formation of resistance. Additionally, PROTACs can overcome existing resistance mechanisms, including target-site and non−target site resistance. This is because PROTACs will be active, even with a reduced binding affinity of the POI to current commercial herbicides, and they are unlikely to be metabolized in a similar manner as current herbicides. Second, there is a big opportunity to rejuvenate shelved herbicide targets, including those that have not been marketed because of selectivity and safety concerns. The tunability of PROTACs offers new ways to address these concerns (e.g., targeting an allosteric site or recruiting a plant-specific E3 ligase). Finally, and perhaps most importantly, PROTACs offer the ability to expand into nontraditional POIs such as structural proteins and transcription factors. Therefore, fully untapped pathways and protein targets leading to herbicidal phenotypes could then become accessible using PROTACs, unlocking a treasure trove of commercial opportunities and applications.

The future of PROTACs in agriculture holds immense promise, offering a revolutionary approach to combating weeds, but also pests and crop diseases. This precise targeting not only will enhance efficacy but also reduce off-target effects, making it a safer alternative to traditional chemistries. Beyond crop protection, there is a bigger opportunity for PROTACs to help farmers grow better, healthier, and more resilient crops in the face of a changing climate. Utilizing plant-specific E3 ligases, PROTAC "sprayable traits" can be developed for on-demand use applications, including drought, heat, and salt tolerance. Overall, the PROTAC technology could thus have the potential to disrupt and reinvent the whole crop protection landscape for decades to come.

Projini's Platform for Discovering New MOA Herbicide Leads Inhibiting Protein–Protein Interactions

Projini is a start-up company founded in 2019 as a spin-off from MIGAL Galilee Research Institute by multidisciplinary scientists with a strong protein chemistry background and industrial experience in drug design. Projini is dedicated to developing new target-site resistance recalcitrant small molecule herbicides that interfere with protein–protein interactions (PPIs) instead of resistance evolution prone herbicides that interact with internal binding pockets (Lu et al. 2020; Nooren and Thornton 2003). PPIs occur in signal transduction pathways and enzymatic cascades where more than one enzyme is required, such as electron transport chains or reactions where enzymes are coupled, and the product of one enzyme is the precursor of the second; where an enzyme has more than one peptide subunit; and where activators/modulators are bound to an enzyme by PPIs. The plethora of PPIs provides many new targets to attack. The main challenge was that

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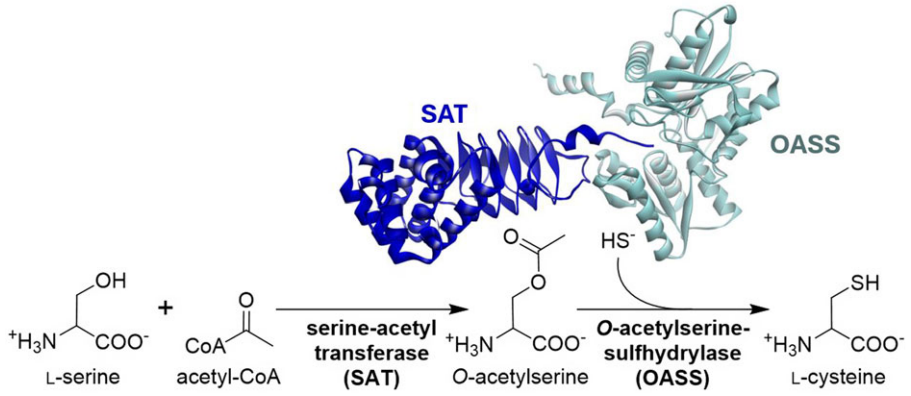

Figure 14. The two-enzyme protein–protein interacting complex catalyzing the conversion of serine to cysteine. The complex is composed of two dimers of *O*-acetylserine-sulfhydrylase (OASS) and a hexamer of serine-acetyl transferase (SAT). Only the binding of the one monomer of SAT whose C-terminal end binds into the binding pocket of one of the OASS monomers is shown. The structures of OASS and SAT were obtained using AlphaFold2. Images and text courtesy of Elad Cohen.

these targets had been considered "undruggable," because PPI sites are often too broad and diffuse compared with classic discrete enzyme pockets that bind most pesticides. Still, the pharmaceutical sector has been developing short peptides, as well as other small molecule drugs, that target PPIs (Shin et al. 2020), and the question had been asked why the herbicide discovery groups have not followed their lead (Gressel 2022).

Target-site resistance is unknown or very rare with quite a few herbicides: the old dinitrophenols, the arsenicals, the carbamates, and the thiocarbamates, as well as the very-long-chain fatty-acid elongase inhibitors, and probably many HRAC Group 0 herbicides. The reason for this that these herbicides probably inhibit more than one enzyme target site. To evolve such multi–target site resistance, a weed would have to simultaneously mutate resistance to each target to remain alive. The expected frequency of resistant individuals when a herbicide has multiple targets is the compounded mutation frequencies of resistance to each target. For example, if a herbicide inhibits two targets, A and B, and the mutation frequency to resistance at target A is 10^{-6} and to target B is 10^{-7}, the initial likelihood of there being a resistant individual in a field is one resistant individual among 10^{13} susceptible ones, an exceedingly low frequency. A herbicide that disrupts PPIs would also require two simultaneous complementary mutations to maintain target enzyme activity while not binding the herbicide, and thus such herbicides would be recalcitrant to the evolution of resistance.

At least two long-marketed herbicides seem to act as PPI disrupters. The auxin mimic herbicides displace a suppressor peptide on the auxin binding site, setting off a cascade of reactions. Likewise, paraquat displaces the protein ferredoxin in binding to P700 in photosystem I in the electron transport chain, disrupting electron transfer. A mutation in ferredoxin would preclude paraquat binding, but then ferredoxin would no longer bind to P700. To retain normal electron transport activity, a simultaneous complementary mutation in P700 would have to occur. Indeed, there are no reported field-fit target-site resistances to auxin mimics or paraquat despite their wide use for more than half a century.

The ag-chem industry discovery groups seem not to have gone the pharmaceutical sector route for a variety of reasons. Herbicide discovery efforts were heavily curtailed during the heyday of the glyphosate era. It also would have been both very expensive and difficult to attempt elucidating compounds that disrupt PPIs. One first had to crystallize the proteins of interest, itself not easy, then perform X-ray crystallography on the proteins and then derive their 3D structures from the complicated diffraction patterns. Only then could one try to determine the structures of the "hot spot" surfaces where the proteins interact most strongly. Experts had claimed that "it will take longer than the age of universe" to compute the 3D folded protein structure from amino acid sequences, but AI changed that with AlphaFold and similar AI-driven software programs that solved the structures of thousands of proteins, replacing the cumbersome process via X-ray crystallography (Arnold 2023; Bryant et al. 2022; Jumper et al. 2021).

Previous work by Projini scientists demonstrated that they could design PPI small molecule inhibitors of plant cystathionine gamma-synthase (Bloch et al. 2021). An additional example is the two-enzyme complex that converts serine to cysteine in a two-step process (Figure 14). The two enzymes must be in physical contact to perform the two reactions.

This enzyme complex does not appear in mammals, which are incapable of synthesizing cysteine, and a specific inhibitor should have no mammalian toxicity. From the 3D structure, Projini determined coordinates of PPI hot spots where the interaction was strongest, and then used Projini's proprietary computational tools to screen virtual libraries of 40 million chemicals in an iterative process: first to 400,000 hits and then to find 250 compounds that should fit, without synthesizing any chemicals, spraying plants, or using greenhouses, all in *silico*... Projini then used two complementary biophysical techniques, isothermal calorimetry and fluorescence polarization, to elucidate the binding affinity K_d and to validate the ability of each molecule to displace the recombinant *O*-acetylserine-sulfhydrylase (OASS) from a serine-acetyl transferase (SAT) peptide. This led to less than 100 leads being tested in vivo, first with an *Arabidopsis* seedling quick test, and then with a cuticle-free common duckweed (*Lemna minor* L.) to ascertain whether, if taken up, a compound is phytotoxic (Figure 15).

The bleaching of the duckweed fronds was expected, as the sulfur from cysteine is used in the synthesis of the iron–sulfur proteins in the photosynthetic electron transport chain. Disruption causes electrons to oxidize chlorophyll and other cellular components. A more complete description of the Projini discovery process and the structure of each lead appears in Dotan et al. (2023). Projini's expertise is in finding leads for new targets, as

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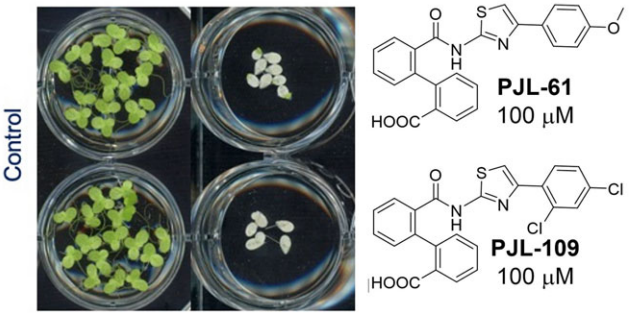

Figure 15. An example of two lead compounds that were phytotoxic to duckweed at 1 wk after sowing four frond-clusters per plate.

shown in the example above and those in the pipeline. Examples of the effects of some of the lead compounds in preemergence assays with various species appear in Figure 16. Such leads go to ag-chem industry specialists for structural optimization and formulation.

As a highly conserved plant hot spot was chosen, it is expected that the lead compounds will be nonselective, unless they or analogues are metabolized by some weeds or crops. Projini can obtain crop selectivity of non-metabolized chemicals by redesigning the protein–protein hot spots to retain enzymatic activity while preventing herbicide binding by changing key amino acids on apposing peptides to generate transgenic or gene-edited resistant crops and improve herbicide selectivity.

In summary, Projini considers efforts to discover single-target herbicides to be passé due to rapid evolution of target-site resistance and has validated its discovery strategy shift to finding herbicide leads attacking PPIs, where two simultaneous mutations are required to evolve target-site resistance. New, sophisticated computational chemistry on docking sites, coupled with computational screening of virtual chemical libraries and elucidation of direct biophysical binding, renders the Projini process much more efficient than random screening.

ENKOMPASS™: A Platform for Target-based Discovery of Novel Crop Protection Chemistries

Enko Chem, Inc. (Enko) based in Mystic, CT, USA, is a target-specific crop protection discovery company that is accelerating the discovery and development of safer, registrable novel crop protection molecules. Incorporating a multitude of emerging research technologies, Enko discovers novel crop protection molecules for target pests faster and more effectively than traditional research and development methods.

Enko utilizes its own scalable, target-based platform, ENKOMPASS™, to efficiently identify novel chemical starting points and rapidly optimize them into lead candidates. The ENKOMPASS™ platform starts with DNA-encoded library (DEL) screening, which identifies novel molecules that act on specific molecular targets, and not on others, from large and diverse pools of chemistry (Gironda-Martínez et al. 2021). Deep exploration of DEL datasets is enabled by Enko's AI-driven data analysis and machine learning capabilities. Enko then uses structure-based design (SBD) to optimize novel chemical starting points rapidly and precisely. Combined, these tools enable rapid optimization of hit chemistries into leads and ultimately product candidates.

DELs are created using pool and split combinatorial chemistry (Lindell et al. 2009) with the synthetic history of each molecule recorded in an oligonucleotide "barcode" that is attached via a chemical linker (Figure 17). The size of a library created using this approach can number in the billions. A screen consists of incubating a specific target protein of interest with a DEL and identifying molecules that bind to the target by sequencing their DNA barcodes (Gironda-Martínez et al. 2021; Peterson and Liu 2023). Billions of sequence reads are produced in each DEL screen, requiring the use of complex algorithms and AI-powered workflows to deliver results that are translated into the chemical structures of binding molecules. Binding molecules are resynthesized and tested for functional activity in biochemical assays and bioassays, with active molecules becoming the starting points for optimization into products.

Enko has created a broad and deep pipeline within its herbicide, fungicide, and insecticide programs, with more than 50% of these programs representing novel MOAs. The success of one of Enko's early projects, the discovery of a novel class of protoporphyrinogen oxidase (PPO)- inhibiting herbicides, screened also for safety to humans and the environment, illustrates the power of the ENKOMPASS™ platform. A DEL screen of more than 120 billion unique molecules, using both the wild-type and resistant variants of the PPO protein, produced 15 distinct chemotypes with activity on the PPO variants used in the screen. Utilization of SBD based on a proprietary, ligand-bound crystal structure guided improvements in in vitro and in vivo activity, leading to testing of a lead candidate in the field ~18 mo from discovery of the original DEL hit. This molecule provides excellent pre- and postemergence control of PPO-resistant populations of *Amaranthus* spp. carrying commonly occurring *PPO2* target-site mutations (i.e., ΔG210, R128G, and G399A) (Barker et al. 2023b). The molecule has now been through 3 yr of field testing with extensive evaluation across 200 field trials in the United States. The candidate molecule has activity against more than 40 key weed species across major crops in both foliar and residual applications, differentiating itself from existing commercial PPO herbicides.

The ENKOMPASS™ platform continues to evolve and expand, incorporating innovations in DEL, data analysis, and other emerging technologies to continuously accelerate the discovery and development of novel crop protection chemistries that set new industry standards in safety, sustainability, and environmental impact.

AI4AI™—An AI Platform for the Discovery of Novel Chemistries

In the age of the AI explosion (Barbosa et al. 2024), Agrematch is a unique company harnessing the capabilities of modern data technologies for the benefit of the agriculture industry. The company leadership, all industry veterans, have a strong belief in AI as the most likely disruptive technology in an industry that still focuses on the use of intensive biological methods for discovery. The current screening methods were developed before the introduction of the computational technology that enables AI to navigate the vast chemistry space still untapped by conventional methods. This paradigm change (Sadybekov and Katritch 2023) could happen only with the convergence of data science and cloud computing that Agrematch harnesses for the discovery and development of novel products that empower farmers globally and contribute to the production of healthier and safer food for consumers.

The company's focus on discovering the next generation of crop protection products is exemplified by its proprietary machine/deep learning compound discovery platform—AI4AI™, or Artificial Intelligence for Active Ingredients. The initial system focused on

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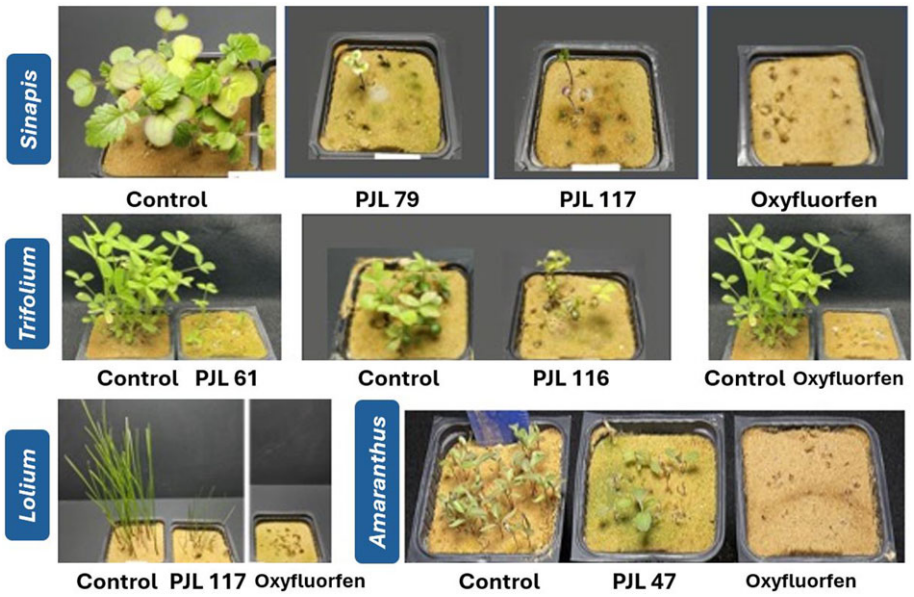

Figure 16. Examples of preemergence activity of Projini leads. The structure of each accession is described in Dotan et al. (2023). The various compounds were applied at rates varying between 1.8 and 2 kg per hectare^{-1}.

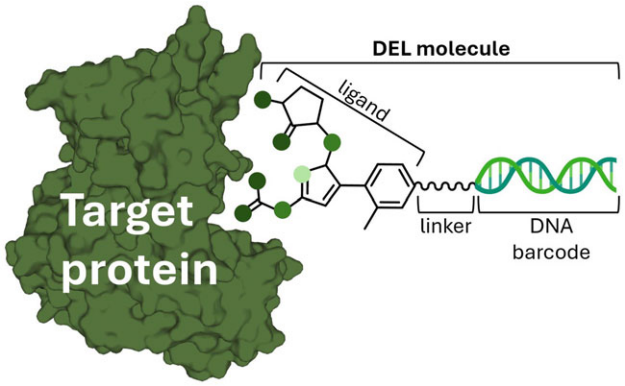

Figure 17. Schematic representation of a DNA-encoded library (DEL) molecule bound to a target protein. A DNA barcode attached to the small molecule through a linker encodes information associated with the specific building blocks that were incorporated into the small molecule during library construction.

addressing critical demands in weed control, resulting in two closely related predictive platforms, each identifying lead compounds for both natural and safe synthetic herbicides.

AI4AI™ is a perfect example of generative AI (Viswa et al. 2024), where the system has been learning the chemical language and its application space to deliver an efficient functional chemistry discovery approach that is rationally designed to cater to the needs of all phases of the product development process from the initial in silico screening. The system excels in accelerated early-stage active ingredient identification, evaluation, and selection by providing predictive in-depth insights into compound characteristics. This methodical, predictable, and fast screening approach enables early decision making, effectively mitigating product development and registration downstream risks.

At the heart of Agrematch's computational platform lies the prediction of compound functions, where the definition of a function is very broad, from specific biological activity such as disease control, to many individual toxicity qualities, and even to the potential cost to produce the compound, to name a few. This is gen-AI, where new insights and knowledge are achieved through a harmonious integration of advanced machine learning/deep learning tools with a massive proprietary database and a rigorous validation process. The system architecture of AI4AI™ has several layers, each providing an interface that enables the efficient creation of many different functional models in many unrelated aspects of the product development cycle. The only common entity is the molecule; hence the method's name: "compound-based." The architecture's effectiveness is evident in its various functional modules already trained and used, encompassing product efficacy (plant stress relief, growth stimulation, weed control, and insect control, to name a few), identification and classification of molecule MOA, many toxicity and environmental safety characteristics, selectivity to certain crops, formulation and delivery, production cost, and many more. This modular computational system ensures a comprehensive and tailored approach to predicting the best compound candidates for a well-defined product concept (Figure 18).

After constructing and training the functional modules within the AI4AI™ system, Agrematch effectively employs these modules to sift through the extensive chemical landscape in search of certain desired functionality. The Lipinski law estimates 10^{60} compounds theoretically capable of biological activity, based on size and chemical composition. This hard to imagine large number of different candidate compounds highlights the immense potential of chemical exploration as well as the need for methods that can sort through vast data in reasonable time. Of this staggering number, only around 100 million compounds have been catalogued, tens of millions have been synthesized, and tens of thousands are being used for human applications, underscoring the vast untapped potential within the chemical universe. The bedrock of Agrematch's technological capabilities lies in its proprietary compound database, boasting more than 6 billion synthetic, a million natural, and as many as desired theoretical compounds. Additionally, Agrematch possesses its own compound generator model, empowering the company to conceive and produce almost any desired compound. The automation tools

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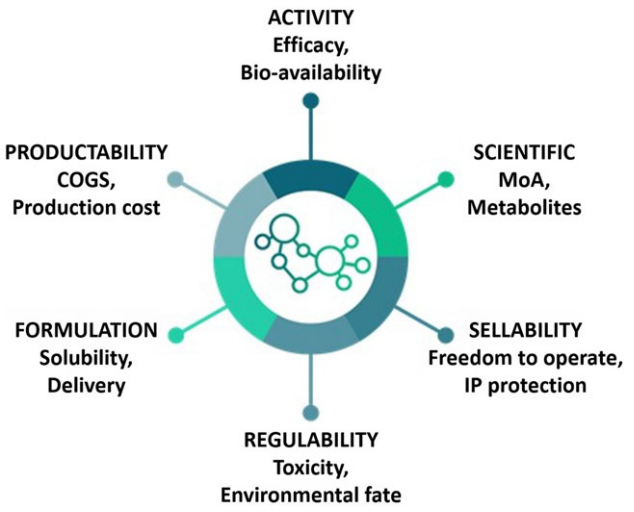

Figure 18. The "compound-based" approach provides early prediction of many critical characteristics and functions for compounds to become products.

integrated into the database support suite play a pivotal role, facilitating processes such as data acquisition, manipulation, standardization, predictive filling, and preparation for resource-intensive applications. This holistic approach emphasizes the platform's capacity to deliver comprehensive and profound compound functional analysis.

In the realm of bioactive chemical computational screening and discovery, a dominant approach was initially developed in drug discovery. This approach assumes a disease agent or a certain protein as a druggable target for the product's active ingredient to interfere with. This target-based approach typically relies on understanding the structure and function of specific target proteins, often utilizing tools like molecular docking to identify potential ligands that interact with these proteins. This method emphasizes the role of proteins as the central players in biological processes, and its success depends heavily on the availability of high-quality protein structures.

Agrematch divorced its process from the need for a target protein and named its methodology the compound-based approach. This method shifts the focus to the chemical compounds themselves, rather than specific target proteins, allowing screening and predicting the bioactivity of compounds based on their inherent characteristics, utilizing computational models and machine/deep learning algorithms. Instead of targeting a predetermined protein, the compound-based approach explores a broader chemical space, considering synthetic, natural, and theoretical compounds.

The advantage of the compound-based approach lies in its versatility and efficiency. Target-based methods are often constrained by the availability of the target protein structures and the knowledge of their full behavior, limiting the scope of the search. In contrast, a compound-based approach allows for a more extensive exploration of the chemical universe, considering a multitude of compounds without being confined to specific protein targets. Agrematch's compound-based approach, powered by its proprietary AI4A™ platform, enables rapid and cost-effective screening, providing a holistic understanding of compound bioactivity and many other critical factors required to become a product. This methodology is particularly advantageous when dealing with novel applications; when detailed information about protein structures is lacking; or when the functionality itself is not protein dependent, as is the case for environmental stability or leaching, movement within the plant, or cost of production. By considering the compounds themselves as the starting point, the compound-based approach offers a more comprehensive and adaptable strategy in the quest for bioactive chemicals.

The Agrematch compound discovery process is initiated with a well-defined research question, supported by training datasets and a compound database. Agrematch's platform showcases the unique ability to design novel compounds in response to specific partner requirements or its own internal pipeline needs. The integration of a validation process adds a layer of rigor to the iterative process, ensuring the reliability and convergence of the computational predictions. In most cases, it takes multiple training iterations that sometimes require new data generation to create a robust, predictive module. The iterative process includes testing the compounds identified by the system for their function, being for example herbicidal activity or MOA, and feeding the results back into the computational system to optimize it (Figure 19).

Agrematch utilizes two proprietary herbicide platforms in house: the Natural Herbicide Platform, designed to create a highly effective and consistent approach to identify herbicides derived from natural sources, and the Safe Synthetic Herbicide Platform, focused on identifying herbicides with new MOAs with optimized safety for crops, humans, and the environment. Notable results from these platforms are the identification of novel inhibitors of

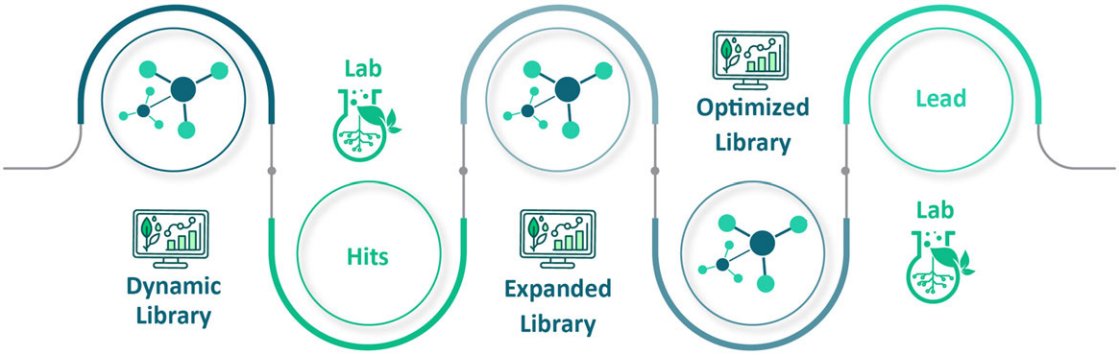

Figure 19. An illustration of the Agrematch platform iterative process of in silico screening, laboratory validation, and feedback to the computational system to generate advanced functional compounds libraries.

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PPO (Barker et al. 2023a) and the ongoing development of a natural herbicidal compound that cannot be divulged yet because of intellectual property issues.

The iterative process of AI4AI™ resulted in a family of synthetic compounds with herbicidal activity and a PPO-inhibiting MOA. In an iterative process of computational prediction, lab validation, feedback, and computational optimization, a screening of an ~1.2 billion compound database resulted in a library of 50 compounds with similar molecular structure. Out of which, 14 compounds had good herbicidal activity in lab assays, which was later confirmed in greenhouse assays. Surprisingly, the MOA classifier algorithm predicted these compounds to belong to HRAC Group 14 (inhibitors of PPO), even though classical similarity methods showed extremely low similarity scores to the structures of known Group 14 herbicide that was used to train the system. Despite the lack of structural similarity with known PPO inhibitors, these compounds indeed acted by inhibiting PPO, causing the expected light-dependent loss of membrane integrity, photobleaching, accumulation of protoporphyrin IX, and inhibition of PPO (Barker et al. 2023a). More recently, a natural compound identified by the AI4AI™ system as having herbicidal activity has been tested in lab and greenhouse assays that confirmed its efficacy as a contact herbicide, surpassing pelargonic acid by 50 times in potency. It boasts a distinctive MOA and stands out for its low production cost.

Summary

The research approaches of the 10 companies described are diverse and creative.

In some cases, products from these companies are already on the market (e.g., Kichawi Kill™), and in others, the product has already been approved by regulatory authorities (e.g., thaxtomin). Some of the discovery technologies described are extremely powerful and offer approaches never taken before by the herbicide industry (e.g., use of PROTAC). These technologies have the potential to vastly expand the molecular targets of synthetic herbicides beyond the fewer than 25 now utilized and to fine-tune these molecules for added crop and mammalian safety. Furthermore, some of these approaches (e.g., inhibiting PPIs, DEL) can produce novel chemotypes that kill weeds by attacking currently used herbicide molecular targets without binding to sites of currently used herbicides. Each of the technologies described in this review has a unique set of attractive features. Some of these new platforms may be particularly useful in providing weed control recalcitrant to evolution of target-site resistance. Furthermore, there are other herbicide discovery start-up companies that are not represented in this review, and the larger, more traditional pesticide-producing companies are diversifying and expanding their discovery approaches. This renewed effort by many research entities is likely to produce significant, new weed management options for farmers to manage their ever-shifting and evolving weed problems.

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Competing interests. The authors declare no conflicts of interest.

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