COMBATING HERBICIDE RESISTANCE WITH OMICS TECHNOLOGIES

New approaches in plant research

Weeds are the single most important reason for crop losses globally, causing high management costs and threatening food security. However, the growing resistance of weeds to some herbicides is making it more difficult for farmers to manage their fields profitably. Bayer is exploring new directions in its search for new herbicides with alternative mechanisms of action and collaborating on this with the start-up company Targenomix.
Protecting crops and controlling weeds: Dr. Anu Machettira investigates the effects of herbicidal test substances on different plant species in the greenhouse.
High tech for new active ingredients: Bayer employee Mario Boecher prepares the pipetting robot for analysis of thousands of herbicidal test molecules in Frankfurt.
Expert round: Dr. Arno Schulz, Dr. Bodo Peters and Dr. Pascal von Koskull-Döring (photo above, left to right) discuss the effects of herbicidal molecule candidates at Bayer in Frankfurt. The researchers mainly work with the model plant mouse-ear cress (Arabidopsis thaliana, photo below).
More than the sum of its parts: Targenomix Managing Director Dr. Sebastian Klie (center) discusses an interaction network of genes with employees and Dr. Pascal von Koskull Döring (right). His interdisciplinary team analyzes the impact of herbicidal treatments on the molecular level.

Weeds can outcompete crops and grow extremely quickly and in such large numbers that farmers are soon unable to recognize their own fields. Field bindweed plants wrap themselves around wheat stalks in China, while ryegrass smothers oilseed rape plants in Australia and steals sunlight and nutrients. Palmer amaranth, meanwhile, can tower over corn plants in the United States. Each individual plant spreads up to 1,500,000 seeds in its immediate vicinity at the end of the season. This weed is vexing farmers in the United States, Mexico, Argentina, and now in Brazil. For farmers around the world, these are devastating scenarios that require them to resort to chemical crop protection agents known as herbicides as a kind of chemical scythe – albeit one that is increasingly proving to be blunt. Nothing controls weeds on such a broad scale as economically as an herbicide.

The number of herbicide-resistant weeds has been growing constantly

Some 250 weed species are now resistant to herbicides and significantly impair the growth of major field crops. This number has been growing steadily for decades, with the result that there are now resistances to 23 of the 26 known herbicidal mechanisms of action. “It’s a global problem that is threatening the world’s food supply,” says Dr. Marco Busch, head of Weed Control Research in Bayer’s Crop Science Division. If farmers had to do without herbicides completely, approximately a third of harvests would be lost because of weeds. The responsible, efficient use of herbicides can reduce this figure to 9 percent. “We simply cannot afford these kinds of losses if we want to feed the nearly 10 billion people predicted to live on this planet by 2050,” says Busch.

In order to sustain the efficacy of existing herbicides, Bayer recommends an integrated approach to weed control. Bayer’s Integrated Weed Management is a holistic approach to sustainable agriculture focusing on managing weeds through a combination of physical, cultural, biological and chemical measures that are cost effective and environmentally sound. “Yet in the long run, we need innovative herbicides to broaden and diversify the toolbox that farmers have at hand.”

One solution is active ingredients that address new targets

One promising approach to this problem is the development of molecules that attack completely new targets in plant cells. “We want to deliver substances that farmers can use against resistant weeds,” says Busch. To this end, Bayer is exploring new ways of supplementing and strengthening the industry’s classic approach to research. Bayer researchers are now collaborating with scientists from Targenomix. This company, a spin-off from the Max Planck Institute of Molecular Plant Physiology in Golm near Potsdam in Germany, has huge expertise in various fields such as genetics, cell biology and biochemistry, and specializes in bioin-
How a plant becomes resistant to herbicides

When herbicides are used repeatedly in the absence of diversity, resistance will eventually develop due to the high selection pressure that drives evolution. On the cellular level, there are several mechanisms that are used by weeds to render herbicides ineffective. In many cases, this is done through degradation of the respective substance by an enzyme so that it cannot reach its site of action intact.
Green hazard: this corn field in Colorado is infested with glyphosate-resistant Kochia. Resistant weeds take away space, nutrients, water and light from crops, reducing their yields.

“We need new active ingredients”

research talked to Professor Lothar Willmitzer about the spread of herbicide resistance. Willmitzer is one of the directors of the Max Planck Institute (MPI) of Molecular Plant Physiology in Golm near Potsdam and is responsible for the scientific work of Targenomix together with Dr. Klie and Dr. von Koskull-Döring.

How dangerous is the increase in herbicide resistance for agriculture?

What I see is threatening. We need a new generation of herbicides that attack new targets within plant cells – herbicides that companies like Bayer deliver. No herbicide with a new mechanism of action has been registered for almost 30 years now, and resistances are on the increase. This is a global problem and we have to act.

So what can scientists do?

There are various options, firstly new chemical products for weed control. Another approach is to change the plants themselves – for example, by making crops less vulnerable to competing weeds.

What is your vision?

Targenomix’ commitment to a bioinformatics-focused approach to this particular problem was and still is very important to me. There is still room in this discipline to shape what it’s all about. In biochemistry and cell biology, by contrast, the scientific concepts are more clearly delineated. Bioinformatics leaves more space in which scientists can spread their wings and follow their own creative impulses. In addition, we are generating data in modern biology more cost-effectively and faster all the time. This trend will continue and will make the tools that we can use to interpret these data all the more important.

Targenomix concentrates on the systems biology approach. How do you assess the potential for non-hypothesis-driven research?

The significance of the systems biology approach – attempting to understand an organism in its entirety – will continue to grow. First of all, we collect a lot of data about our test object and then derive a hypothesis from the data as a whole. So we end up with the hypothesis, while conventional research begins with one. At the end of the day it’s all about describing the biological system better and I personally believe that we will then be able to understand it better too. There are without doubt others in the research community who see things differently. Their view is, just because we know what parts a car contains doesn’t mean we understand how it works. One thing I’m certain of, is that these new approaches and methods will lead to the generation of more knowledge about how plants work. Some may have underestimated the time aspect: research is by definition an open-ended affair because there is no finish line to cross.
Herbicide resistance: worldwide problems

Weeds that are resistant to herbicides and thus endanger harvests are spreading all over the world. In the United States, for example, 156 uniquely resistant populations have been identified in weeds – more than in any other country.

The actual research and development process for new substances capable of keeping weeds in a field in check and thereby safeguarding harvests initially begins on a small scale – in a 150 mm² microtiter test plate with 96 wells for individual biological tests. Each well contains weed seedlings which are sprayed with different test substances. Other test plates hold seedlings of a different weed species. Bayer researchers use these young plants to test thousands of molecules from the company’s own substance library. This molecular repository comprises several million vials containing different test substances, with new ones being added daily. “We examine their effects on plants using a high-throughput screening process, in which many of the process steps are automated and carried out by robots to increase efficiency,” explains Dr. Pascal von Koskull-Döring, coordinator of the Targenomix collaboration project at Bayer.

Effective substances are tested on larger weeds and crops

Promising active substances are taken one step further in the process and are tested on larger plants grown in soil. In addition to increasing the spectrum of weeds tested, the researchers can then investigate whether the substances have an effect on the crops they are supposed to protect. If the screening process produces a candidate that looks promising, it must pass strict toxicity and environmental safety tests before it can progress to field tests. “From the laboratory into the field – that’s the most difficult step,” says von Koskull-Döring. Once taken outside the greenhouse or growth chamber, the candidates are also exposed to environmental factors, which can lead to different results. The researchers do not normally analyze their test plants at the
Avoiding resistances - safeguarding harvests

Herbicide resistance is a highly complex problem. “There is no simple, one-size-fits-all solution,” says Dr. Bodo Peters, head of Project & Product Support in Weed Control Research at Bayer’s Crop Science Division. He and his team at the Weed Resistance Competence Center – WRCC for short – in Frankfurt are therefore taking several different approaches. Their main focus is research into resistances, the management of resistant weeds and the development of effective, integrated weed control strategies for farmers. “In this way we can help farmers when they are confronted with herbicide resistances,” says Peters. The WRCC receives samples of resistant weeds from farms all over the world, investigates the type of resistance, and then searches for solutions. This close proximity to the actual situation in the field is essential for active ingredient development as well. “Otherwise we might develop herbicides that are not what farmers need at all,” says Dr. Marco Busch, head of Weed Control Research. The researchers have significantly increased the capacities of their chemistry laboratories to enable them to find new substances more quickly. In Frankfurt, some 40 researcher positions have been created with financial support from the Grains Research and Development Corporation (GRDC), an Australian cereal growers association, as part of a cooperation with Bayer that began in December 2015. Eleven of these researchers are Australian and New Zealander post-docs who will contribute their experiences from their home countries, which are among the areas most heavily affected by the resistance problem. “These additional scientists will help us find new, resistance-breaking active substances,” explains Busch. The collaboration with the startup company Targenomix will also help. After all, “This is a problem that we can only resolve together with farmers, plant researchers and all involved stakeholders,” says Peters.
Plants on a minute scale: Bayer employee Tanja Zupritt programs a robot that processes weed seedlings in ultracompact wells (photo above). Lab technician Heiko Jung inspects plants that are growing under constant conditions in a climatic chamber (photo below).
Omics technologies for new active ingredients

In Frankfurt, Bayer researchers treat the model plant mouse-ear cress with selected herbicidal test substances. The effects on the molecular level are examined by the experts at Targenomix in Golm. To this end, they employ a systems biology approach and use state-of-the-art omics technologies to determine which plant-specific biomolecules are affected by the candidate substance.

The plant material is ground in a mill. The samples are mixed in a reaction vessel with tiny beads and then accelerated to a speed of approximately 1,000 oscillations per minute. “We’re really splattering the cells,” says Klie.

This tissue homogenate contains what the scientists are ultimately looking for – the molecules inside the cells. “These molecules originate in a wide variety of cellular processes. They include genetic information (DNA), the form of DNA that is used to convey genetic information (RNA) to the proteins which are the final link in the flow of genetic information influencing a cell’s metabolism and numerous metabolites – the biomolecules that take part in almost all cellular processes,” explains Klie.

Different classes of molecules necessitate using different methods to study them. For example, once the plant experts have isolated the transcribed genetic information (RNA) from the homogenate, they send their samples to a service provider which then sequences all of the RNA molecules it contains. This information is reconstructed to give the researchers information about which genes are specifically up- or down-regulated in the plant after herbicidal treatment as computational analysis can identify...
Elucidating life with systems biology

To understand the processes involved in life, it is not enough to describe individual biological mechanisms. What is needed is a holistic view of the organism to understand how these fit together. This is the objective of systems biology. It differs fundamentally from the current experimental approach to science used in biological research. Usually, a research project begins with a hypothesis which it then attempts to confirm or disprove. Systems biology turns this approach around; here, the data and the patterns they make lead the scientists to their hypotheses. Experts also call this non-hypothesis-driven research. Systems biology aims to observe all molecules in a cell and their interactions over time. It also describes interactions between cells that are locked together in different tissues, as well as their interactions in organs and the organism as a whole. This mammoth task can only be resolved with a combination of state-of-the-art molecular biology, mathematical modeling and the computing power of supercomputers. Researchers from the most varied of disciplines – mathematics, bioinformatics, chemistry, biology and IT – are therefore working closely together in this project.

New hypotheses derived from comparisons between treated and untreated plants

They look for differences between treated and untreated plants. If the researchers discover any peculiarities, they may be able to develop an idea about which cellular molecule a molecule binds to and in which process it intervenes. At the heart of the research in Golm is Targenomix’s central database, which points the scientists in the direction of new hypotheses to explain how compounds can affect plants. This approach is called systems biology, because the computer searches through huge quantities of data at different levels of organization within the cell, looking further for irregularities and inconsistencies to then derive hypotheses about the entire plant system. “These complex interrelationships are almost impossible for humans to grasp,” says Klie. Once the researchers have identified a molecule from Bayer’s substance library that is likely to be based on a novel mechanism of action, they go back into the laboratory. “The initial hypothesis is not.

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For better crop protection: Targenomix employee Norma Funke (photo above) prepares mouse-ear cress seedlings for omics analyses in Golm. Dr. Marco Busch, Dr. Axel Trautwein and Dr. Pascal von Koskull-Döring (photo below, left to right) are coordinating the collaboration with Targenomix on Bayer’s side.
“Countering the danger posed by weeds with diversity”

Numerous farmers all over the world are struggling with herbicide-resistant weeds. How do you regard the problem?

The biggest problems are facing the major large-scale farms. The situation is particularly difficult in the United States, Canada, Brazil, Argentina and Australia – the main grain exporting nations. These countries generally grow glyphosate-tolerant crops such as soybeans, corn and cotton. These fields are treated solely with glyphosate, and increasing numbers of weeds are becoming resistant to this herbicide. Fifty million hectares of arable land are infested with glyphosate-resistant weeds in North and South America alone.

How did this happen?

Because of over-use of glyphosate without diversity. It’s simple evolution biology. Wherever there is strong selection pressure – in this case overwhelming use of one herbicide – resistances will quickly evolve. Exclusive use of one herbicide across large areas without a sufficient diversity of weed control methods will always lead to resistances, no matter where it is done.

What are the most promising strategies to avoid or minimize resistance?

In a nutshell: diversity, diversity and more diversity. Herbicides are an outstanding tool for dealing with weeds. But they must be part of a larger strategy: only a combination of various weed control methods can be successful in the long term. There is no one solution. Chinese small-holders have very different needs than an Australian farmer growing wheat on 4,000 hectares of land. Nonetheless, both need a balanced mix of different herbicides and other weed control strategies so that they can work sustainably to produce good yields.

What approaches are you taking in the AHRI?

We’re trying to show farmers the benefits of a diverse weed control strategy. To this end, we are looking for tools that we can use to ensure sustainable herbicide use. One example is “harvest weed seed control”, in which we attempt to ensure that as little weed seed is left in the field at the harvest as possible. This greatly reduces weed numbers in these fields. This method is finding increasingly widespread use in Australia and being trialed in Canada and the United States, which we are naturally very pleased about.

Where do you see agriculture 20 years from now?

By that time we will have a projected 9 billion people to feed. That is something we can only do with highly productive, sustainable grains farming. Weed control must be an important part of the strategy, or we will lose yields that we urgently need. I expect herbicides to still be an important tool in our arsenal against weeds in 20 years’ time. That’s why we have to learn how to use them sensibly. We will have to continue to fight to make sure that our fields remain productive. We can and must succeed in this; we need human brains and the right technologies.

enough on its own, we have to demonstrate that the herbicide really does bind to the assumed target molecule,” explains Klie. Start-up companies like Targenomix investigating the basic principles of a mechanism of action as a service provider are a relatively rare phenomenon in plant research. As Klie says, “This model is much more common in the medical and pharmaceutical industry.” But their successes have proven to him and his team of experts from the most varied of disciplines that this approach works. His geneticists, biochemists, plant physiologists and bioinformatics specialists have already discovered several mechanisms of action for a number of herbicidal molecule candidates since 2014. “We have only a few organizational structures – initiative and constant dialog are our philosophy. We discuss our work everywhere: at the coffee machines, over lunch and of course in the laboratory,” says Klie. The working relationship of the team in Golm is all about mutual trust. And the collaboration with Bayer is likewise trust-based. “We meet twice a year to share our scientific findings and to discuss the current status and our plans for the future,” says Trautwein. The future of the collaboration over the coming years is assured – the cooperation agreement does not expire until 2019. “The collaboration has gone very well so far, with high scientific standards and a trusting and cooperative relationship,” says Trautwein. It takes between 10 and 15 years on average for an active ingredient to make it all the way from research to the market as a commercial product. Until then, farmers will have to make do with the tools they currently have at their disposal. But Bayer and Targenomix will continue to feed the research pipeline with new candidate substances.