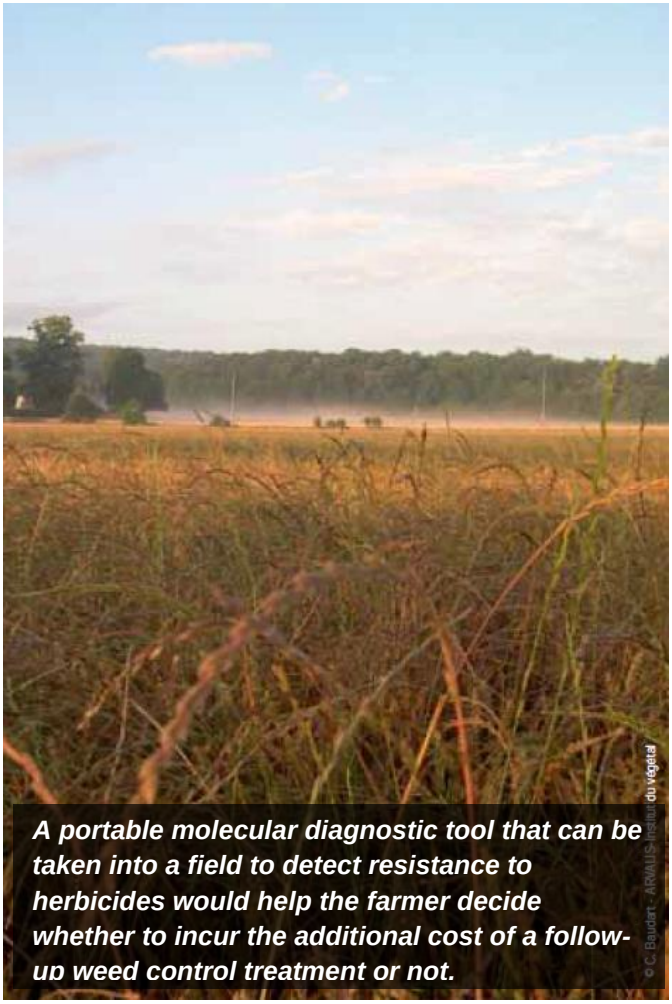
IDENTIFYING
weed resistance directly in the field



A portable molecular diagnostic tool that can be taken into a field to detect resistance to herbicides would help the farmer decide whether to incur the additional cost of a follow-up weed control treatment or not.

A research project is aiming to produce a prototype for a cheap "DIY" tool that will help farmers diagnose ryegrass resistance to herbicides directly in their field. To achieve this, it will involve both the farming and participatory biology (or biohacking) communities.

Weed management has become many arable cropping systems' main challenge, partly because of the growing resistance of weeds to herbicides. This has been compounded by tighter regulations. Many herbicide substances have been removed from the market in France and elsewhere in Europe. The radical changes that need to be made to cropping systems in order to help farms pull back from the brink of a deadlock situation, are difficult to implement. The idea behind the DIY-LOL (Diagnostic-It-Yourself-LOLium) project is that a personal diagnostic tool to identify resistance directly in the field, will help farmers make decisions and manage weeds sustainably.

A tool to support difficult decision-making

In 2015, 247 weed species had become resistant to 157 different herbicides worldwide. Ryegrass (Lolium), in particular, is showing strong resistance to acetolactate synthase (ALS) inhibitors, a type of herbicide used mainly for cereals. And yet, yield losses due to the presence of weeds in crops are high (-7% for wheat worldwide). Less effective herbicides often lead to the use of follow-up chemical treatments. According to a survey carried out by ARVALIS- Institut du végétal and the Chambres d'Agriculture, those additional treatments have cost farmers in the Paris basin over €120/ha. They threaten the objectives of the Ecophyto plan aiming to reduce the use of plant protection products.

Cultural control methods (mechanical weed control, rotation, etc.) are effective but rarely used because of feasibility issues, time constraints, or even because of fear of the consequences that a change in practices might bring. The development of a resistance diagnostic tool would help farmers make operational decisions, both during the season (follow-up treatments, choosing a mode of operation) as well as when planning rotations (for example after a diagnosis on stubble, sowing barley after wheat will help, if the level of grass weed resistance is such that it will be impossible to control them) or in the longer term, with a view to changing the cropping system. This type of tool must be cheap, reliable and quick and easy for farmers to use themselves.

An innovative prototype

The first step in this project is to develop a molecular test to diagnose ryegrass resistance to sulfonylureas (ALS inhibitors). This field is advancing very rapidly. Several innovations are currently opening up some interesting application possibilities. New sequencing technology is slowly but greatly simplifying access to the genes known to carry resistance. In addition, a wide range of approaches and technologies are revolutionising the concept of diagnosis and genotyping on the spot, as the sample is taken. This technology is going to make it much easier for the farming world to access molecular diagnostics. Finally, ongoing miniaturisation in molecular biology is facilitating the emergence of portable systems that can be taken into fields.

Faster identification of mutations

Traditional resistance tests are effective and reliable, but results take between 5 and 18 months, which is far too long to help a producer make a decision. Conversely, molecular diagnostics helps to identify the presence of resistant weeds very quickly, by detecting gene mutations that lead to resistance (insert). The project will assess several molecular diagnostic techniques. The dCAPS method is an effective and reliable technique which uses leaf DNA to identify, in just a few days, the mutating plants that are resistant to ALS inhibitors. However, it does not give any details on the nature of the mutation. Recently, some methods based on DNA isothermal amplification managed to eliminate the need for heavy equipment. One of them, the LAMP (Loop- mediated isothermal amplification) method is becoming more readily accessible and used to study crop bio-aggressors. In about 30 minutes, it amplifies specifically the target gene to reach numbers that can be seen with the naked eye (turbidity, colorimetric indicators) or with easily available equipment (gel electrophoresis, spectrophotometers, fluorescence…). Already used to detect many yeasts, fungi and viruses, it can be adapted to identify ryegrass resistance.

« In the long run, this tool's development will have a much wider impact than just on weed management or control in a winter cereal-based

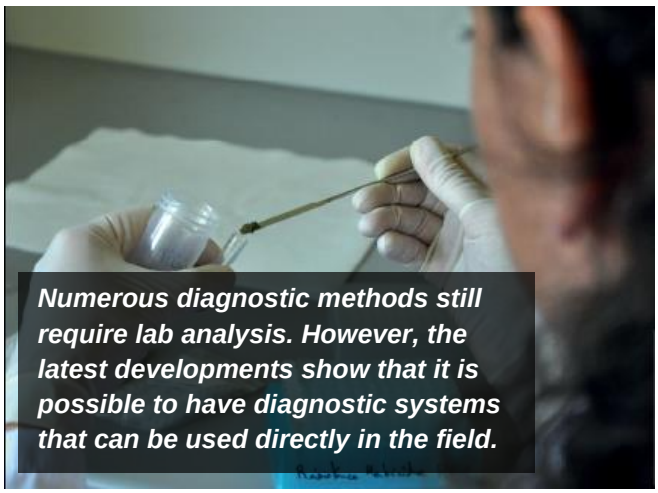
A project overlapping several sectors

Many of the current methods involve using lab services; however, the latest developments show that it is possible to have diagnostic systems that can be used directly in the field. As project coordinator, ARVALIS- Institut du végétal will ensure that the bio-technological tools that are being developed meet the need for solutions that can be used in a farming environment. The Institute will involve, among others, its biotechnology laboratory in the development of molecular diagnostic techniques capable of detecting ALS inhibitor resistant ryegrass. The weed control specialist team will supply the samples and required data.

SupBiotech (a further education establishment specialising in biotechnology) students will base themselves at ARVALIS to develop the prototype of the DIY-LOL molecular diagnostic tool. Once created, the prototype will be tested on the Boigneville Digiferme® experimental farm, with a view to duplicating and improving it further.

INRA will develop molecular diagnostic techniques to detect NTSR. At the same time, the company ADNid, specialising in this type of diagnosis for the agricultural and food industries, will deal with target gene monitoring using a new sequencing technology.

Working and support sessions for students will be organised by La Paillasse, an association of laboratories dedicated to the implementation of innovative collaborative projects, including biohacking ones (insert).



Numerous diagnostic methods still require lab analysis. However, the latest developments show that it is possible to have diagnostic systems that can be used directly in the field.

What is biohacking?

Also called biopunk, participatory biology, DIYbio or "garage biology", this movement was born less than a decade ago in the United States.

Research is taking place outside of the administrative constraints and highly technical environment of official labs, in a spirit of collaboration and sharing.

It is based on technological progress and the fact that new equipment is easier to use and cheaper than before. All the community members have free access to the results.

Scientists keen to share their practices and research interests with "amateurs" who are able to bring a fresh look and ask unexpected questions, are often at the root of a participatory lab. However, DIYbio labs agree on a common charter that provides a methodology framework and sets ethical limits.

In traditional resistance tests, weed seeds are sown, then sprayed with herbicide to ascertain the mortality and survival (resistance) rates.

A potentially multifunctional tool

From a methodological point of view, this project is one of the first French initiatives linking participatory biology and agriculture around a common interest. The development of a molecular diagnostic tool that can be used directly in a field to detect ryegrass resistance to ALS inhibiting herbicides may also help to diagnose resistance to other plant protection products, for other weeds than ryegrass, and in other crops than cereals. In the long run, the solution developed as part of this project will therefore have a much wider impact than purely controlling weeds in winter cereal-based rotations.

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« Ryegrass resistance to ALS inhibitors is essentially due to two mutated codons **»**

Ryegrass resistance

There are two broad resistance categories: target-site resistance (TSR), where mutations in the targeted gene, confer resistance to the plant, and non-target-site resistance (NTSR), much less known, involving a wide range of mechanisms, and therefore of genes.

Ryegrass TSR is well known. The mutations conferring resistance to ALS inhibitors mainly concern two positions (or codons) in the gene in question: out of the 527 plants taken from samples gathered in France by ARVALIS that were tested and analysed through gene sequencing over a period of three years, 27 % presented TSR, 94 % of which were at the P197 codon position and 6 % at the W574 codon position (figure 1). It is therefore those two mutations that the DIY-LOL project's diagnostic tool will need to identify.

NTSR has been the focus of several recent studies suggesting that monitoring a restricted number of genes should be sufficient to reliably establish the existence of a NTSR in ryegrass.