

The rapid response technology



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DR ADRIANA BOTES

A problem vexing the brightest minds in crop production – as new chemistry becomes more difficult to discover and commercialise, can pests and diseases be tackled by other means? *CPM* investigates the emerging science striving to achieve just that.

By Rob Jones

In a hi-tech laboratory near York, something innovative is brewing – a project that could change how growers manage their approach to crop protection.

Star of the show is *Gluconacetobacter diazotrophicus* (Gd) – if this sounds familiar, that’s because it’s already attracted attention for its ability to convert atmospheric nitrogen into plant-accessible ammonia.

It achieves this feat from within the plant’s cells – in leaves, stems and roots – providing a steady stream of nitrogen throughout the growing season.

Having isolated and patented a particularly efficacious strain of Gd, Az19, available to UK growers as Encera, it’s these same plant-colonising characteristics that firm Azotic Technologies seeks to utilise in a new project.

Dr Adriana Botes, research and development director, says this is because Gd has a second string to its bow beyond nitrogen fixation. “We’re exploring the ‘programmable plant’ concept.

“The premise – already proven – is that we can silence certain

genes in the plant or in the pest, to offer protection or resistance.”

This so-called ‘gene silencing’ is already being used in human health, where it’s deployed to alleviate disorders such as cystic fibrosis, and is also under investigation as a potential cancer treatment. In crops, researchers originally focused on the transgenic route – around the turn of the century, US growers could purchase genetically-modified potato varieties resistant to the potato leafroll virus.

INFLUENCING GENES

Then around 20 years ago, the naturally occurring biological process of RNA interference (RNAi) was discovered. RNA is chemically similar to DNA, the nucleic acid molecule that carries the genetic instructions for all living organisms. Its molecules influence which genes are suppressed or expressed.

“In turn, another molecule is associated with this gene expression process, and that’s double-stranded RNA,” explains Adriana. “Plant scientists realised they

could tailor a specific dsRNA molecule that when applied to plants, could silence an essential gene within a target pest.

“There it would inhibit a critical biological process, resulting in death, thus reducing the pest population as would a traditional insecticide.”

The first foliar-applied, dsRNA-based bioinsecticide was approved by the United States Environmental Protection Agency in 2023. The active ingredient, ledprona, targets the Colorado potato beetle, silencing a gene that produces a vital protein.

Without this protein, the beetle stops eating and dies. Recognised as a new



Inconspicuous yet efficacious

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- ▶ mode of action by the Insecticide Resistance Action Committee, ledprona offers farmers control of a pest that accounts for global crop losses of more than \$500M.

"It's called spray-induced gene silencing (SIGS)," says Adriana. "Without doubt it's a more sustainable approach to crop protection and pathogen control.

"With tailored dsRNA – which can be produced by large-scale fermentation – growers could have new options for insect control, fungal pathogens and plant viruses, with minimal off-target effects.

"Such a new mode of action could replace many chemical-based pesticides and avoid the cycle of resistance against new actives. It also provides an alternative to creating and using transgenic plants, GMOs, which are restricted in many parts of the world."

Despite its benefits, Adriana says SIGS faces a major obstacle: its delivery method. "As a foliar spray, its effectiveness depends on uptake of sufficient dsRNA. It has to penetrate the waxy cuticle, the cell wall and the membrane before it accesses the plant cell's own RNAi machinery.

"Even then, it won't move through the plant systemically, so while it's good for insect pests feeding above ground, it's of limited use for pests that target roots."

But, here enters Gd. Could its innate ability to colonise a wide variety of crops be used to produce and deliver dsRNA, so that it's delivered within the plant itself?

Adriana believes so. In fact, it's why Azotic's new project is supported with a £500,000 grant from the UK's Advanced Research + Invention Agency (ARIA) as part of its 'Programmable Plants' opportunity space.

"Gd is like a Trojan Horse, delivering dsRNA right into the plant cell. Then we use the plant as the fermentation vessel to produce more Gd and dsRNA. That's more effective, more efficient and less expensive.

"What's more, dsRNA isn't the only molecule that modified Gd can produce," she points out. "Enzymes and peptides, for example, are powerful biostimulants and many exhibit antimicrobial properties too.

"But they're both very difficult to apply foliarly. Around 99% of a peptide application is degraded before it gets inside the plant."

Adriana points out that the attraction with Gd, besides its ability to colonise the plant, is its versatility. As well as targeting plant pests and diseases, dsRNA can also alter plant traits without permanent genetic modification.

"We're talking the ability to simultaneously transform all cultivars of a crop using a single strain of Gd engineered to express one or more genes. We could improve crop quality, productivity, or resilience against abiotic stresses such as drought, salinity or even heavy metals."

Ahead of the ARIA project is a smaller, Innovate UK-funded study with partner FERA. It's testing efficacy of modified Gd strains on the root-knot nematode *Meloidogyne incognita*.

"It's a devastating pest – it attacks more than 3000 plant species and causes losses of up to \$70Bn each year. It's a great example of a pest that can't be tackled by SIGS, nor the other variations of gene-silencing currently available such as transgenic plants or virus vectors.

"How would you ever re-engineer all the crops it affects to create dsRNA-producing versions? You can't, but, you can do it simultaneously with Gd."

PROOF OF CONCEPT

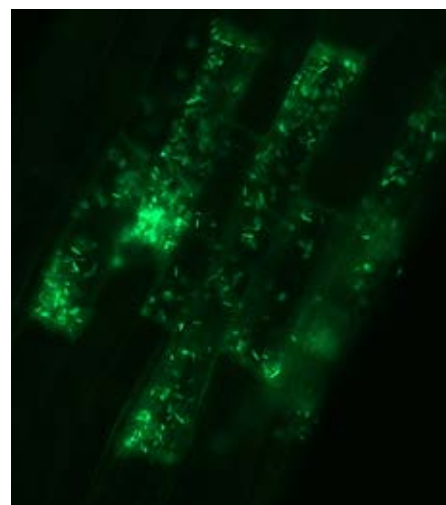
The project with FERA finishes in January and should confirm how a foliar application of Gd colonises the plant's roots to produce dsRNA that silences a specific gene in *M. incognita*. But the bigger prize is the proof of concept that will come from the three-year ARIA study.

In partnership with the University of Durham, the team is using *Arabidopsis kaleidocell*, a laboratory plant modified to fluoresce. By measuring the intensity of this fluorescence after the plant's been treated with dsRNA modified to silence the fluorescent gene, Ariana can compare a SIGS treatment against Gd.

She expects first-stage results (due in spring 2026) to confirm that not only does Gd work to suppress the fluorescence, but that it does so for far longer than SIGS (more than six weeks, compared with seven days). Further project stages will confirm Gd's ability to produce and secrete peptides within the plant cell of rice, corn, wheat, potato and tomato, followed by work to demonstrate that Gd is as effective as CRISPR techniques in altering plant phenotypes.

This last point is highly topical in view of this year's observed breakdown in the YR15 yellow rust resistance gene, notes Adriana. "The gene features in several important varieties and had been seen as an important line of defence since its discovery in the 1980s."

However, Gd could potentially create a 'repair patch', she says. "This could increase a variety's resistance again by targeting a specific gene. The same



Florescent marker

Seen here under a microscope, Gd has colonised the root cells of tomato, seven days after inoculation, producing green fluorescent protein (GFP) as a marker.

Photo: Azotic

goes for a disease like potato blight; once you know which genes in which varieties are responsible for conferring resistance, you can put those into Gd and bring resistance to other cultivars."

Adriana believes even emerging pests and diseases could be tackled relatively quickly. "It's a rapid response technology – you'd be able to create an effective control if not within the same season, then certainly by the following one.

"And for existing pests and diseases, we can develop protection where no effective solutions currently exist."

The intention is to build a portfolio of modified Gd strains, each offering a different property: nematode control, an anti-fungal, insect control, activity against phytophthora, and so on. "You could apply these as required through the season, perhaps starting with a seed treatment followed by later foliar applications.

"But each one of these strains would still provide Gd's characteristic N-fixation; growers will be getting a dual-action product."

However, Adriana says the main stumbling block is how regulators will perceive the modified Gd strains. "These gene expressions happen only when Gd colonises and proliferates within living plants, but there's no survival outside the host plant or in progeny – the microbe isn't present in harvested seeds.

"Nevertheless, the bacteria will be engineered, and so we must engage with regulators to encourage the regulatory landscape to 'keep up' with new biotechnologies and their potential; it's untapped potential." ●