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“We’ve been looking at things from a different angle – from inside out.”

# A marriage of biology and chemistry

**Bioscience in practice**

Often biology and chemistry are considered separately but in nature some were designed to work together. *CPM* takes an in-depth look at how biology can work with very specific biostimulants to help improve nutrient-use efficiency.

By Lucy de la Pasture

**There’s a saying: “You don’t get results by focusing on results. You get results by focusing on the actions that produce results.” It’s a mantra that applies just as much to improving the nutrient use efficiency of crops, says Unium director John Haywood.**

“There’s a lot of talk about improving nutrient use efficiency, particularly as fertiliser prices have rocketed, and using biostimulant products in a very general way is quite prominent in that discussion at the moment. We’ve been looking at things from a different angle — from inside out.

“It’s something we’ve been working on for a long time and we’ve approached the problem by first looking at the fundamental plant physiology and then at how this can be influenced.

“It’s from that point that we construct biostimulant products rather than the common approach, which is the other way

around — finding an effect in the field and only then trying to figure out how it’s working.”

So what are the actions that growers could take to help improve nutrient-use efficiency? Many biostimulants work by reducing the impacts of abiotic stress and enhancing root production, which helps plant roots scavenge for nutrients, says John.

## Targeted approach’

“But there’s a more targeted approach using a combination of biology and biostimulant products in a way that supports one another. It starts with using biology to fix nitrogen from the atmosphere; followed with a targeted biostimulant to help plants assimilate the N; and then a signalling compound to enhance the production and transport of plant sugars — to the benefit of both the plant and biology.”

Fellow Unium director Dr Nigel Grech takes up the story, wearing his hat as a researcher but also as a farmer himself in California. He explains that although adopting biology is germane to farming systems, in the past it has been one of the great paradoxes in agriculture — of great benefit but also variable in its effects and with results that aren’t reproducible.

“There’s a big interest in non-rhizobial microorganisms that can fix nitrogen from the atmosphere. Data from legumes suggests that approximately 30-50% of the plant’s N requirement can be supplied by inoculation with nodule-forming, rhizobial nitrogen-fixing organisms in a climatically good year. This drops to 25-30% N in a poorer one — reflecting inherent biological variation. But until recently there was nothing

comparable for non-legumes.”

The advent of molecular biology changed the game and enabled scientists to identify plant symbiotic N-fixing endophytic microorganisms and begin to unlock the mysteries of the complex plant microbiome, he explains. This research has been led by Prof Sharon Doty and her team at the University of Washington. Thirty years since she started, Sharon isolated a group of strong nitrogen-fixing endophytes which colonise the vascular system and interstitial spaces in plant tissues — roots, shoots, stems and leaves.

“The fact that these particular strains live within the plant tissues is important as it means they’re subjected to lower environmental stresses and oxygen levels. This gives them an advantage over other microbial-based products coming onto the UK market, such as ones based on Methyloths — containing bacteria from the genus Methylobacterium. ▶



*John Haywood explains that there’s a more targeted approach to improving NUE, using a combination of biology and biostimulant products in a way that supports one another.*



*The endophytes in Tiros live within the plant tissues which means they're not subjected to the higher environmental stresses and oxygen levels which could inhibit N-fixation, says Nigel Grech.*

► “Methylotrophs are mainly surface-dwelling in the phylloplane — the leaves and stems of plants — and preferentially utilise single carbon molecules as a food source. Living on the leaf means they're subjected to greater environmental stresses such as high levels of UV and oxygen (which are generally inhibitory to the nitrogen fixing enzyme systems these microbes employ).

“Similarly, colonisation of the internal tissues of plants also reduces exposure of

the Tiros bacterial strains to oxygen levels inhibitory to their N-fixing enzymatic,” explains Nigel.

Unium has collaborated with US company, Intrinsyx, to take the Doty research into the field and launched an endophytic seed treatment, Tiros, in the UK in 2020. It's gone on to become the market-leading biological seed treatment in 2021 on small grains.

## Diverse strains

Tiros contains two strains of endophytic bacteria which Nigel explains helps to reduce the variability that is often a feature of biological products, particularly ones based on a single strain. “We selected N-fixing bacterial endophytes rather than fungal endophytes so that there wouldn't be any adverse effects from fungicide inputs.

“The diverse strains are generalist, able to utilise a broad range of sugar sources and are strongly endophytic plant symbionts, which are also facultative — meaning they respire using oxygen but are able to switch to fermentation, if necessary,” he adds.

This approach has produced an endophyte package that works and with

results that are reproducible — as shown in the extensive trialing before Tiros' launch and the data continuously being captured by Unium.

“Because the endophytes are genotyped, we can be confident there are no nasty surprises introducing these into fields. We're able to trace them in the environment and in plants using qPCR. That's an important part of being able to quantify biology.”

Endophytes are present and well conserved in natural systems, but modern breeding/phytosanitary practices wipe them out in commercial germplasm and therefore in commercial farming situations, explains Nigel.

“Like our own gut microflora, that mediate so many processes critical to human wellbeing, plant microbial endophytes evolved to be symbionts and a healthy microflora in plants has a tremendous positive effect on many plant processes, one of which is assisting is nitrogen assimilation.

“This ongoing relationship between biology and the plant is important because with more nitrogen being brought into the system through fixation, this has to be supported by taking up more other nutrients.

## Healthy microbiome has a crucial role

Like many farmers who've made the decision to adopt regenerative practices, James Walgate thinks a lot about what he's trying to achieve. He's thirsty for knowledge to further his understanding of how to grow crops and the biology that can support them. Assimilating information in his DNA.

Even before James began his journey into conservation agriculture (which merged into regen) six years ago, he was at the heart of on-farm innovation — hosting trials for Dr Chris Green of Crop Management Information (CMI), whose work really connected farmers and research and in so doing gave an unparalleled insight into what works in a real-life situation.

Farming 668ha in the North Lincolnshire Wolds, his rotation is diverse and lengthy to help develop the soil microbiome that will underpin his crop production. With soil at the heart of regenerative principles, James has a no-till strategy and makes good use of cover crops, with a 300 head flock of breeding ewes just being established on the farm to better utilise both cover crops and his permanent pasture.

“The ewes are ‘Easy Care’ — Lleyn cross Exlana — and will lamb outdoors in May. The idea is they will require minimal assistance at lambing, with ideally just 1% needing help at lambing and a further 3% soon after (pairing up, mis-mothering etc). They'll fit in really well with our rotation.”

The sheep will join the pigs which 'bed and

breakfast' at the farm, suppling valuable FYM to return to the land which is supplemented by green waste compost brought onto the farm.

Going hand in hand with James' regenerative journey is a desire to reduce the farm's reliance on synthetic inputs — with organic regen being the Holy Grail, though he accepts he may not quite get there. One of the inputs he's trying to reduce is ammonium nitrate.

“We're looking at nitrogen use efficiency very hard and we're applying any bagged nitrogen little and often to help increase NUE. If you're applying 222kgN/ha in a conventional system and NUE is only 40% then that means only 90kgN/ha gets into the plant. By increasing the NUE to 60% then you only have to apply 150kgN/ha to get the same amount of N into the crop.

“We believe that by switching away from high levels of N, crops won't be predisposed to the pest and disease problems associated with it. A plant also needs eight times as much water to be able to use the AN applied at conventional levels and we're looking at alternative nitrogen sources to mitigate this effect,” he explains.

James hosted some of the early trials for CMI which looked at different strains of endophytes, so he was already pretty confident in the integrity of Tiros when it became commercially available. This now forms the foundations of his approach to reduce reliance on AN fertilisers and help make



*James Walgate says he's very wary of replacing one type of input with another one as the overall aim is to use 'less' rather than 'more' products from a can.*

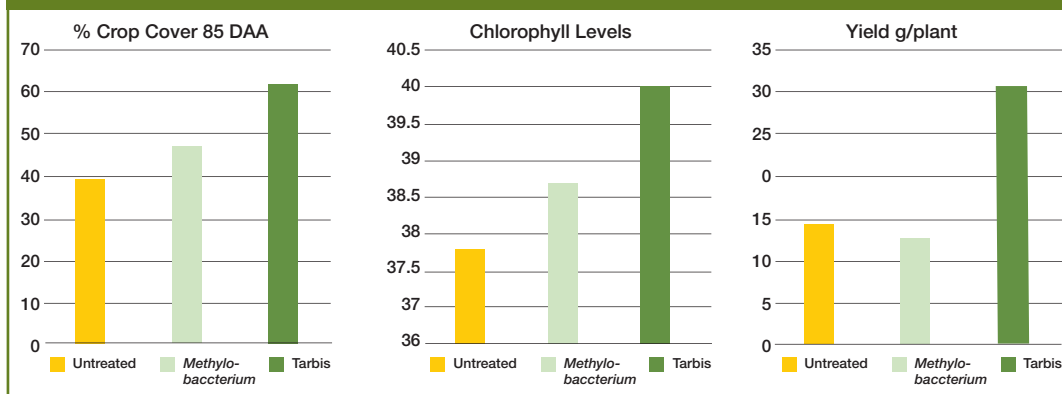
the plant more efficient.

“We're very wary of replacing one type of input with another one as our aim is to use 'less' rather than 'more' products from a can,” he says.

That means there has to be some well-structured reasoning behind his approach, which he's found to be the case when supporting the endophytes in Tiros with Twoxo and ultimately T6P, where again James witnessed a lot of the early trials work on the farm.

He believes that what he's doing is right because it's backed up by the farm's yield results. “We're finding our yields are similar to our neighbours using a conventional farming system but we're saving £150/ha in costs,” he concludes.

## Leaf dwelling vs colonising endophyte strains



Source: Unium trials, 2021

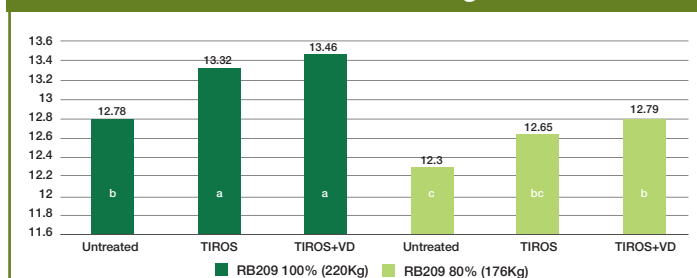
## Nitrogen dose response trial



The effects of foliar Tarbis (endophytes) on winter wheat yield across a nitrogen dose response trial in Suffolk.

Source: Unium, 2020

## Effect of Tiros under different N regimes



Source: Newark, 2020

The strains in Tiros augment this process and they also help modulate the plant's responses

to abiotic and biotic stresses."

For growers who have missed applying Tiros to their seed,

there's good news. A foliar version, Tarbis, will be available this spring, he says. "The liquid formulation is pretty tough, it's comfortable in the tank with agrochemistry and once the endophytes are applied, they go into the plant for life."

## NUE support

The Tiros/Tarbis formulations contain a prebiotic to support the endophytes (probiotic) while they colonise plant tissues — a technology which uniquely marries biology and chemistry together to help provide consistent results, says Nigel.

The second element to support nutrient-use efficiency is Twoxo — which contains the plant metabolite 2-oxoglutarate (2-oxo) in combination with L-PGA.

The 2-oxo molecule stimulates nitrogen uptake and the system becomes somewhat self-sustaining because it regulates a key assimilation bottleneck in plants — the

glutamine synthetase pathway, explains Nigel.

Crucially, it stimulates the whole plant system to perform at a higher level, yet it still remains in nutritional balance. "The key is that if you elevate N, it's such a critical system that you have a cascade effect whereby other elements are acquired by the plant to maintain nutritional balance. In this system you have an incredible opportunity to improve N-efficiency through a plant-mediated system, but unless there's availability of other nutrients, particularly micronutrients (which often have important metabolic functions), then you won't get maximum benefit," he explains.

Nigel explains that being such an important plant enzyme, 2-oxo integrates well with endophytes to help plants assimilate the nitrogen being fixed. "It's a beautiful and elegant marriage of effect when the two are put together," he says.

The ultimate element of the Unium package to help improve NUE is the signalling molecule T6P — Trehalose-6-phosphate — which amongst other beneficial effects, impacts sugar metabolism and transport in plants, says Nigel.

"T6P is the plant's natural signalling mechanism for modulating and transporting sugars, something that's been well reported in published peer reviewed papers, with much of the research being led by UK institutions.

"When N is fixed it's married to carbon atoms, from CO<sub>2</sub> fixation via photosynthesis, and forms amino acids. Endophytes and 2-oxo both increase this carbon sequestration and much of this carbon goes into the production of sugars, which need to be translocated around the plant based on need. T6P plays a very important role in this process. That means that all three products work in concert to support one another — what's good for plants is also good for the endophytes — completing the circle." ■

plants a natural boost for life. Twoxo XL helps the plant assimilate that nitrogen, increasing its efficiency and 3-Alo T6P supports carbon sequestration and signals to the plant to enhance sugar metabolism and transport, which benefits both the crop and the endophytes.



## Bioscience in practice

As the chemistry toolbox continues to shrink, a mesmerising array of new bio-solutions are coming to market, offering a range of benefits and complementary additions. Evaluating just how effective they are, and where they're best placed can be tricky.

In 2021 CPM teamed up with Unium BioScience to open the science behind these innovations. In this second series of articles we explore how bioscience can be

utilised in the field, building on our understanding of the physiological processes and trials data. Above all, these articles give the grower an inside view on some of the exciting opportunities biosolutions offer in the field.

This first article in the series looks at how NUE can be improved by using biology and supporting it with specialist biostimulants. In the Unium programme, Tiros provides endophytes which fix nitrogen, giving