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# Endophytes – a new frontier?

## Innovation Innovation Insight

A new seed treatment that enables crop plants to fix nitrogen from the atmosphere is available to UK growers this autumn. *CPM* charts the new technology's journey from academia, to outer space and now to the field.

By Lucy de la Pasture

Look into the night sky and you may see a fast-moving streak of light. Some 408km above the Earth, it marks the path of the International Space Station as it orbits the planet at 18,000mph. On board is a technology that's being tested in preparation for man's planned visit to Mars. It's a development which will enable crops to be grown in space flight but it's also set to benefit UK growers, who will be the first to have access to it in the form of a new endophyte seed treatment, *Tiros*.

The story began along the banks of the Snoqualmie River in western Washington State, where two pioneer plant species thrive — poplar and willow, explains Prof Sharon Doty of University of Washington.

“Originating from high alpine snow melt from the Cascade Mountain Range, the Snoqualmie River has a very low nutrient content, less than 0.5mg/l total nitrogen. The rocks and sand deposited by the river also supply little to no nutrients, yet poplar and willow colonize this raw substrate readily.

“Vigorous growth in the absence of the

macronutrient nitrogen (N) isn't possible, indicating a likely symbiotic partnership with N-fixing bacteria. The symbioses of nodulating plants (legumes or actinorhizal plants, for example) with N-fixing bacteria (rhizobia and *Frankia*, respectively) has been well characterized, but neither poplar nor willow form nodules,” she comments.

That seemed to indicate another mechanism was at work and, more recently, a similar association of N-fixing microorganisms with non-nodulating plants has been brought to light, says Sharon.

### Microbial nitrogenase

“N-fixation activity was confirmed in wild poplar samples taken from the Snoqualmie River site, both directly through the tracking of  $^{15}\text{N}$  incorporation and indirectly through the acetylene reduction assay. These results were also supported by the presence of microbial nitrogenase genes in the wild poplar microbiome.”

This important discovery was just the beginning and through in vitro culture, Sharon's team isolated a wide range of symbiotic microbes. These include endophytic *Rhizobium tropici*, yeasts, and a variety of other microbial species sourced from within wild poplar and willow branches.

Plants have had associations with micro-organisms since they first appeared on earth. But it's only recently that the plant biome has been studied and few plants have had their total biome understood, so Sharon's research has opened the door for the development of new tools to support crop production.

Endophytes are microorganisms that internally infect living plant tissues without causing any visible manifestation of disease

and live in mutualistic association with plants for at least a part of their life cycle, she explains.

They've been found in all plant families but different species form associations with different strains. If a range (consortium) of superior endophytes is made available to crop plants, then this can have beneficial effects.

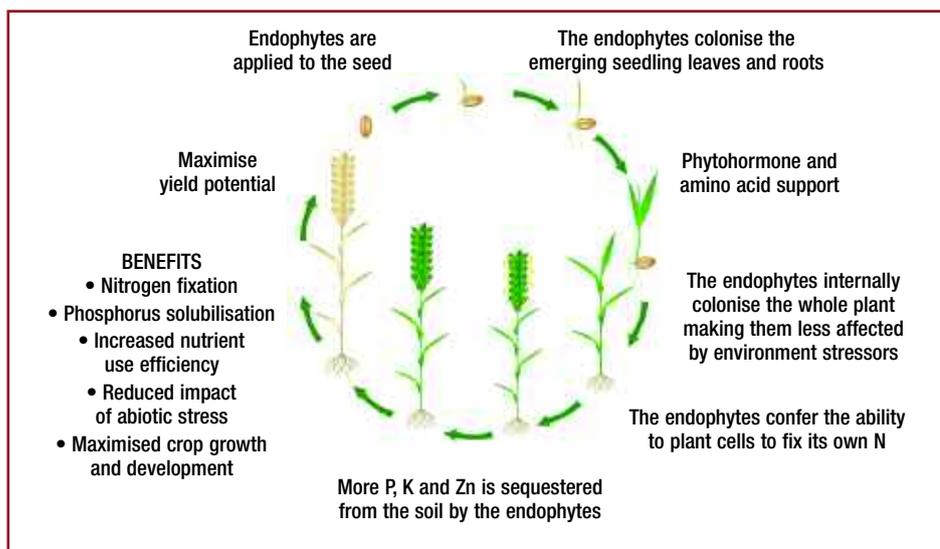
“Over the past two decades of characterising these strains, we've determined that some of these microbes make nitrogen and phosphorous bioavailable, increase photosynthesis and water use efficiency, as well as promote plant growth, yields, and health under drought and nutrient stresses,” she explains.

Sharon has screened the strains she's characterised to select a 'Phase A' consortium, which includes microbes with these traits, as well as some with the natural ability to produce plant hormones — including auxins for enhanced root growth and gibberellins for shoot growth.

“Genomic analysis has also indicated



Endophytes that can fix nitrogen from the air were discovered in willow and poplar growing along the banks of the Snoqualmie River in the USA.



Endophytes applied to seed bring benefits for the entire life cycle of the crop. Source: Unium Bioscience

capabilities for production of (R,R)-2,3-butanediol and acetoin — volatile phytohormones known to increase plant growth and drought tolerance. Microscopy of plants inoculated with endophytes carrying plasmids for the production of fluorescent proteins has shown that, in the absence of nodules, symbiotic microbes are likely able to colonize the vascular tissue of their host plant, which would allow for systemic interactions with their host,” says Sharon.

Sharon’s academic research has been taken up by Intrinsyx Bio (USA), based at the NASA Ames Research Park, who are commercializing the technology because of the potential endophytes confer to crop production systems and for space travel. “These endophytic microbes cause crop plants, grasses, ornamental plants, and trees to fix nitrogen directly from the atmosphere into ammonia, which helps improve nutrient use efficiency, supplements

synthetic fertiliser requirements, and increases leaf chlorophyll and food crop quality,” explains Dr John Freeman, plant biogeochemist and chief science officer at Intrinsyx Bio.

## Genome sequence analysis

Intrinsyx Bio first screened and then commercially deployed the phase A strains after confirmation of their safety by full genome sequence analyses at the University of Washington. Since then, both field trial results and reduced nitrogen fertiliser studies have shown impressive yield increases across a wide variety of broad acre and speciality crops.

“Our endophytes have been sent as seed treatments to the International Space Station for growing broccoli and rice in outer space,” he says, explaining the company supports crop experiments being carried out in the Space Station programme.

The potential for seed endophytic bacteria is underestimated and under-researched, believes John Haywood, director of Unium Bioscience, the company responsible for bringing this technology to the UK as a seed treatment for commercial growers.

“It’s a real breakthrough that will help growers manage their carbon footprint by ▶

## Light years ahead

When the late Dr Chris Green, one of the industry’s most thought-provoking researchers, spoke enthusiastically about a new technology he was trialling at CMI, growers paid attention. It was Chris that first introduced Cambridgeshire farm manager Russell McKenzie to the concept of endophytes.

The biostimulants field remains a little cloudy and Russell very much subscribes to Chris’ philosophy that farmers should make data-driven decision processes based on proven research. For that reason he likes the Unium approach, where the technologies are peer-reviewed, extensively trialled and the economics for the farmer is transparent. He also likes that Unium can explain the science behind a technology but also why it may not work in some instances.

So when Tiros was on offer last autumn, Russell changed part of his seed order to put 12ha of RGT Saki in the ground alongside his usual single purpose dressed seed.

“We’ve seen emergence effects before with Take Off and Vibrance Duo and it’s hard to quantify how important it is, but I thought it would be interesting to have a look. In our no-till system, emergence isn’t always that fast and as drilling gets later, then speed of

emergence becomes more important.”

Having now seen Tiros in action, Russell believes it’s an exciting development for a number of reasons. “The autumn drilled Saki (with Tiros plus SPD) was through the ground three to four days quicker than our standard treatment (SPD). But really interesting was the effect in an early Feb planted field, where we put Tiros around the headland of a field drilled with another variety.

“It was almost as if they had been planted at different times. The speed of development was light years ahead, with the Tiros-treated plants having two leaves when the rest of the field was just emerging,” he says.

Russell has been struck by the long-lasting effects of the endophyte treatment. “Improved nutrient scavenging and utilisation was something John has spoken about and we’re seeing longer leaves and more efficient use of nutrients through the season.”

Tissue tests are regularly taken to provide a snapshot of the nutrient status and the Tiros-treated plants have consistently had a better balance of nutrients with less deficiencies, he explains.

“We are still applying some trace elements but not as many because the crop appears to be less



Russell McKenzie has been struck by the long-lasting effects of the endophyte treatment, with the nutritional status of plants better even late in the growing season.

limited where Tiros has been applied.”

The farm is participating in Bayer’s Field View and as a result NDVI images have been available. “The 40m strip of Tiros-treated crop has shown up consistently as a greener stripe down the field during the first three weeks of May, so it’ll be interesting to see how this translates into yield at harvest, it potentially looks very exciting, but final performance will reveal the true results.”



John Haywood says Tiros is a real breakthrough that will help growers manage their carbon footprint by increasing the nutrient-use efficiency of crops.

► increasing the nutrient-use efficiency of crops. The selection process has identified the most efficient endophyte for fixing atmospheric nitrogen and for sequestering phosphorus from the soil. The data is compelling and will lead to improvements in how crops are fertilised.

“These plant-microbe associations are known to be beneficial for germination and seedling establishment. Seed endophytic bacteria are found naturally in these early plant growth stages, but Tiros provides a combination of highly functional strains that wouldn’t normally be present to form additional associations with the host plant.”

John describes Tiros as a ‘back-up generator’ that’s running all the time the plant is growing. When nitrogen and other nutrients are limiting, the endophytic bacteria within the plant tissues provides a solution — helping the plant keep going and then recover once conditions become more favourable.

## Innovation Insight

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Further proof of concept has been gained under UK conditions over the past three years in glasshouse studies at the University of Nottingham, independent replicated small plot work, as well as larger farmer evaluations.

So what can growers expect from the Tiros endophyte treatment? Endophytes can promote plant growth naturally through improved nutrient acquisition, phytohormone production and stress tolerance, leading to fast germination and establishment, explains John.

## Prioritise resources

“Very occasionally the seed will prioritise resources into its roots rather than shoots if environmental or root conditions are sub-optimal. This actually benefits the plant once it emerges and trials have shown crops are faster to establish and better able to withstand drought conditions.

“Trials have shown Tiros significantly increases the biomass (shoot and root) over untreated and other seed treatments and is long-lasting. In winter barley trials in 2019 the difference in biomass (compared with untreated) became evident 60 days after treatment and continued to increase at a far greater rate in the following months.

“The larger leaf area has also been found to contain more chlorophyll, improving the photosynthetic capability of plants and ultimately increasing yield. Over 30 trials the average yield response was 0.58t/ha (range -0.19t/ha to 1.84t/ha) and we calculate Tiros is likely to give a 90% chance of return on investment from the data.”

Endophytes also improve nutrient use efficiency (NUE), so leaves have a higher accumulation of nitrogen. This is the case for other nutrients, including phosphate and potash, which may be particularly useful where phosphate is locked up in the soil,” explains John.

“Our initial work with Tiros

indicates a 20% reduction in nitrogen use is possible, without a significant loss in biomass occurring. Further nitrogen response work is being carried out this season to explore this potential.”

By virtue of all the testing he carries out for different companies, Dr Steve Rossall has a unique insight into the seed treatment technologies available. He believes the endophyte treatment looks like a real breakthrough in biological seed treatment technology.

“The opportunity Tiros provides to improve nitrogen and phosphorus nutrition is very exciting for the industry. In our work, it more than compensated for the reduced nitrogen treatments (as ammonium nitrate or urea) when compared with the control,” comments Steve.

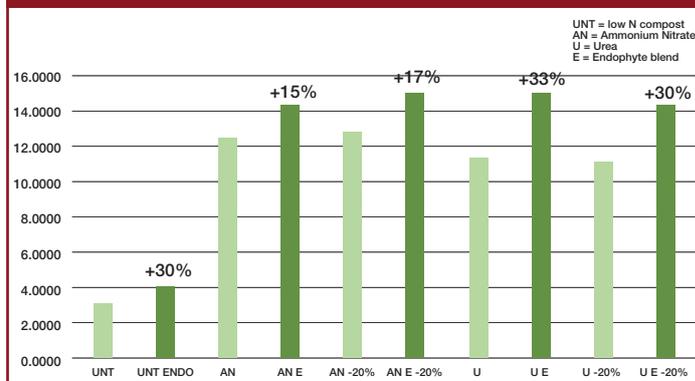
“An interesting fact is that endophytic bacteria with beneficial characteristics can be

selected by the plants and these may differ between varieties,” adds John.

It’s an attribute that has attracted the attention of plant breeder, Bill Angus. “We’ve been working with these products for three years now and they look very promising. As wheat breeders we recognise that good genetics needs to be supported on farm with good husbandry. This starts with the seed and we see a big future for endophytes as part of this strategy. We’re keen to see if we get variety interactions, which will be really interesting,” he comments.

“I’m aware of the benefits seen by adopting endophytes in some crops — results to date from three years of trials on wheat look exciting. Breeders have struggled to find reliable genetic differences in wheat with regard to nitrogen use efficiency — endophytes could be a solution,” concludes Bill. ■

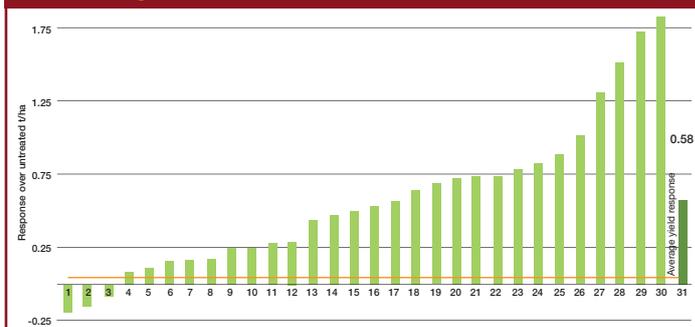
## Effects of endophyte treatment on Nitrogen Use Efficiency



Trials on winter wheat showing improvements in NUE from Tiros when different nitrogen forms are applied.

Source: University of Nottingham, 2019.

## Yield response from Tiros



The average yield response in winter wheat trials in 2018-2019 was 0.58t/ha, with a breakeven of 0.04t/ha required for a return on investment.

Source: Unium Bioscience, 2020.