Metabolite reclaims its throne

"2-oxo stimulates the plant to do what it would do naturally, but it's doing it faster and for longer."

Technical
Bioscience insider

Scientists have found a natural way to trick the plants own metabolism to use and assimilate more nitrogen and fix more carbon from the atmosphere without any negative effects. *CPM* reveals the plant metabolite responsible and its relationship with the 'imposter molecule'.

By Lucy de la Pasture

The backstory to pyroglutamate (PGA), used as a seed treatment, reveals it is in fact an 'imposter' molecule subsidising for a natural plant metabolite — 2-oxoglutaramate. The metabolite was discovered by Dr Pat J Unkefer and the late Dr Thomas J Knight while working at Los Alamos National Laboratory — a prestigious scientific establishment in the United States that has been responsible for landmark innovations such as the nuclear reactor, medical isotopes and fuel cells to name but a few.

Pat has spent most of her career working on nitrogen (N) utilisation. Now retired from Los Alamos, she's still very much involved in seeing her discovery become a commercial reality for the first time as Twoxo, officially launched in the UK at CropTec in November last year.

What does the science say?

The story begins when Pat was investigating a plant pathogen that secreted a toxin that inactivated an enzyme (glutamine synthetase) catalysing ammonia assimilation into glutamine. It was important because all N entering plant metabolism passes through this step, she explains.

"When alfalfa plants were inoculated with the pathogen they grew extremely well and nodulated heavily. We began to investigate what was happening at the metabolic level."

Pat believed the toxin had another site of action besides the glutamine synthetase enzyme and that it structurally mimicked another molecule. This insight guided her to identify 2-oxoglutaramate (abbreviated as 2-oxo), produced by the ω-amidase pathway, and she found that it exists in equilibrium with its cyclic form, named 2-hydroxy-5-oxoproline (abbreviated as 2-hop). She believed these important metabolites play a key role in monitoring nitrogen assimilation in plants.

We began to synthesise 2-oxo and apply it to plants under controlled conditions and we observed greatly improved growth," explains Pat.

Because 2-hop has stereoisomers (an identical atomic structure but different spatial arrangement) which exist in equilibrium, they can't be separated. In order to find out which isomer was active, Pat looked for a similar molecule in size and structure to study and that's where PGA became part of the story.

"L-PGA started out as a research tool and provided the evidence that the 2-hop form of 2-oxo was active," she explains. "PGA is structurally similar to 2-oxo and is a stable molecule with L and D isomers which are separable. We found both

Pat Unkefer identified the plant metabolite 2-oxoglutaramate, and found it plays an important role in monitoring nitrogen assimilation in plants.

Nigel Grech explains that when 2-oxo is applied to plants, it upregulates N uptake and there's a cascade effect whereby other elements are acquired by the plant to maintain nutritional balance.

isomers were active so we could infer that both stereoisomers of 2-hop are also active.

From Science to Bioscience

When Pat started her work, the cost of making 2-oxo was prohibitive. "The first synthesis cost $1,000,000/Kg and the process was problematic. For this reasonL-PGA became the commercial technology, though we always knew the effects were greater with 2-oxo," she explains.

Dr Rodolfo Martinez, organic chemist at Los Alamos, cleverly devised a method to make it cost-effectively. This has enabled the technology to become commercially available for the first time as the Unium product, Twoxo.

"From a scientific and practical viewpoint, the thing to know about this molecule is that it stimulates nitrogen uptake beyond what the plant would normally be doing. This system becomes somewhat self-sustaining because the 2-oxo has increased the N uptake by the plant. Once N uptake has been stimulated, it turns on a critically important set of genes — nitrate transporters — and increases the levels of an enzyme which reduces nitrate to ammonia and hence N-assimilation through the glutamine synthetase pathway," explains Pat.

"The increased N uptake and assimilation in the plant turns on over 1000 genes, including those responsible for CO_2 uptake, protein synthesis and photosynthesis, as well as uptake of other mineral nutrients. By increasing the N-uptake, 2-oxo upregulates the whole plant to take advantage of the greater N."

"That's the beauty of this system, where the metabolite acts to stimulate N-uptake," she adds. "The plant is already geared to maintain all the nutritional stoichiometry that it requires. 2-oxo stimulates the plant to do what it would do naturally, but it's doing it faster and for longer so there's more of it. In other words it stimulates the whole plant system to perform at a higher level, yet it still remains in balance."

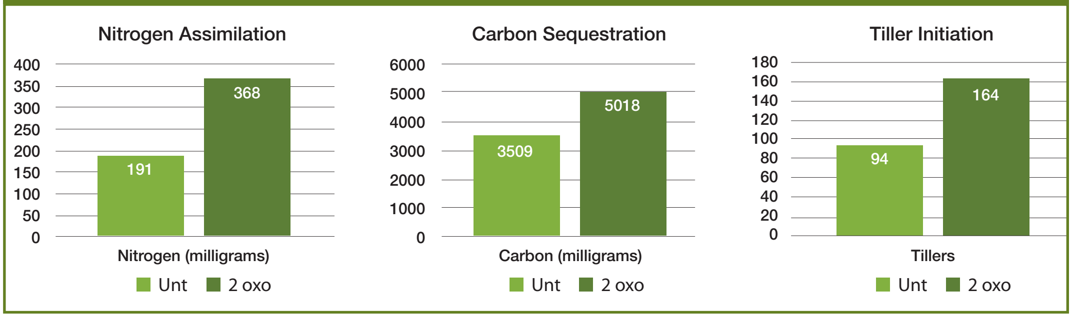
Dr Nigel Grech, previously a researcher at the University of California and director of Unium Bioscience, has been working with Pat and her team for many years to better understand the effects of 2-oxo on crop plants.

"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.

"We've seen over the years working on this, that when you look at treated versus untreated plants the actual footprint of the nutrient levels in both sets is the same, except in the treated they're all elevated. Even though the primary target is N-metabolism, there's an uptick across the whole nutritional spectrum."

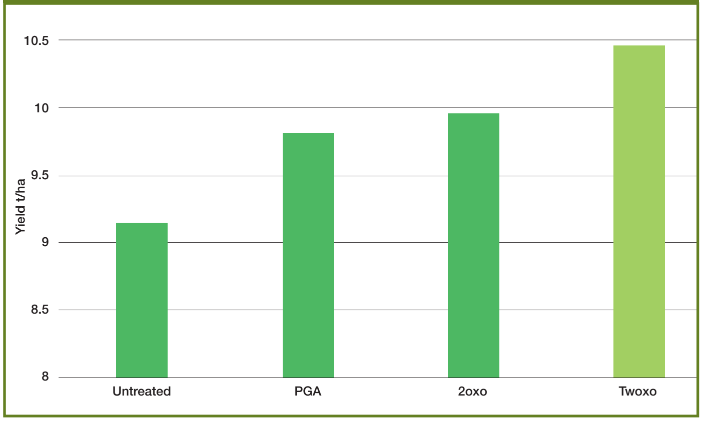
A consistent effect observed in treated plants is that they have elevated leaf proteins and ►

Effect of 2-oxo on winter wheat



Source: Unium, 2021

Performance in the field



The Effects of 2-oxo /PGA in Oct-drilled winter wheat.

Source: Envirofield trials, Suffolk; 2018

Effect on components of yield

Treatment	Yield t/ha	Ears/m^2	Weight of Ear	Grains/ear	TGW	Kg/Hl	Hagberg	Protein	Screenings 2mm
Unt	8.38	124.6	1.89	32.81	57.6	73.6	193	12.81	0.73
Twoxo	8.69	141.8	2.08	35.98	57.8	76	199	14.08	0.77

Spring wheat, variety KWS Cochise, in tramline trial conducted by Niall Atkinson in Lincs.

Source: Unium, 2021

- increased chlorophyll levels which reflects the increased light harvesting by the plant and is consistent with their faster CO_2-fixation. Nigel explains that 60% of leaf proteins are associated with photosynthesis.

Proving the concept

2-oxo has been verified in many glasshouse and field trials in both the US and UK. It's been found to out-perform L-PGA because it's the functional signalling molecule that regulates one of the most important pathways in plants.

And that's one of the key messages about 2-oxo, highlights Nigel. "It isn't a functional analogue, it's a natural molecule augmenting the plant's own system. Pat has also discovered that when you put 2-oxo and L-PGA together, you get a synergy and the combination significantly upregulates the efficiency of plant systems.

"Adding L-PGA creates an effect beyond that the 2-oxo can produce by itself," confirms Pat. "The 2-isomers in 2-oxo aren't at the optimum ratio in nature, they're in equilibrium. What we've found by working with the imposter molecule (both L & D stereoisomers of PGA) is that we can find the optimum ratio and that's where you start to see the synergy."

One of the remarkable things about proving 2-oxo in the field has been its consistency, says Nigel. "Field work is about failure most of the time, most product concepts don't work but this one did. If 2-oxo is applied correctly at the right phenotypic windows then you see effects across monocotyledonous and dicotyledonous crops, from seed treatments and exogenous foliar applications.

"The earlier you apply it, the more pronounced the effect. Plants set themselves up in terms of their genetic potential very early on. Once the plant is established there's a predetermined programme they will go through in terms of N-utilisation," he explains.

"Plants grow exponentially so the earlier you make an intervention in any exponential process, the bigger the impact," adds Pat. "The more cells, the bigger the seedling, the faster out of the ground and so on."

"Genetic potential is affected by environmental factors," continues Nigel. "As such, N-utilisation and efficiency can be impaired by stress conditions on the plant. Plants evolved in a system with very low levels of exposure to nitrogen but in fertiliser events we're exposing them to very high levels of N and they simply cannot cope, so they down regulate N-transporter uptake systems, root growth is impaired (as nitrate inhibits auxin metabolism) and there's a loss of unused N that goes on to environmentally pollute — as well as an economic loss to the farmer.

"What 2-oxo does is to switch on N-uptake and assimilation mechanisms, making the plant more nutritionally efficient and root growth is unaffected as the plant thinks it's a bit short of N," he notes.

Pat explains that it's possible to measure NUE in a number of ways. "We can measure the amount of N that goes into the plant and we see increased yields without diluting the protein in field trials. Also we can look at above ground biomass and we've seen this is elevated in both glasshouse studies and field trials. The larger leaf size of treated plants is because the plant is more aggressive in taking up N.

"This is a natural system that stimulates N-uptake beyond normal when a plant is given 2-oxo. Essentially it tricks the plant's system using its own mechanisms to uprate the plant to become more vigorous and healthier," she comments.

"Stressed plants release aromatic compounds from parts of amino acid biosynthesis, and we've seen that those compounds aren't produced in a very vigorous plant, so there is less predation in a very healthy plant," adds Pat.

Pioneers in the field

Lincolnshire technical agronomist Niall Atkinson was able to get first-hand experience of using Twoxo in the field in 2020. The concept of using a plant metabolite to improve the way the crop makes use of N and to help it fix more carbon has a good fit

Bioscience insider

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, however.

This series of articles opens a window on the science behind these innovations. *CPM* has teamed up with Unium BioScience to explore the background, unravel the physiological processes and provide analysis on the results of trials. Above all, these articles give the grower an inside view on some of the exciting opportunities biosolutions offer in the field.

Unium have worked with 2-OXO and L-PGA active ingredients for over 15 years and are really excited to now bring the most effective formulation to market at an affordable price. It's an exciting step forward for Nitrogen Use Efficiency as it can be used as a fertiliser coating, seed treatment or foliar spray.

Niall Atkinson is keen to explore the potential of Twoxo to help plants utilise more nitrogen and reduce the overall levels of synthetic fertiliser applied.

with his journey towards a more sustainable, conservation agriculture.

"Reducing the carbon footprint is one of the big issues with synthetic N fertilisers. As we move towards Net Zero, there's a possibility we may well get a cap on N-usage so anything that can reduce our dependency on fertilisers is a good thing," he says.

Niall is using cover crops routinely in the rotation but hasn't yet felt brave enough to make substantial cuts in N inputs, though he will do when better nutrient cycling starts to kick in on his soils.

"Trialling products like Twoxo on farm is the best way to look at them, it's important to understand how they're working so that we can get the best out of them."

A trial (24m x 300m) was set up using alternate tramlines in a consistent part of the field, planted with spring wheat, variety KWS Cochise. Twoxo was applied at 1.0 l/ha at early stem extension, just prior to the main dose of N.

When monitoring the crop from the ground Niall says it was difficult to see any differences between treatments but when it was surveyed from above, the GAI (growth area index) was 7.9% higher in the treated plots and it was clear to see the crop was denser. The weather caused harvest to be delayed by a month, so the crop had shed some heads by the time the combine got to it. In spite of this it yielded approaching 9t/ha, but it could have done better, believes Niall.

"We recorded a yield increase of 3.6% in the Twoxo treated area. The grain quality characteristics were also elevated — with 14.08% protein compared with 12.81% in the untreated and a specific weight of 76kg/hl compared with 73.6kg/hl in the untreated. The first year's results look very promising, though it is just one trial in one year," he comments.

"We will be looking to evaluate Twoxo further, particularly to find the answers to how much we could cut back N usage, if at all, without compromising net margin to reduce our carbon footprint." ■