

Biology steps in

“It sets a good example for what we can expect to see from biologicals going forward.”

Innovation Innovation Insight

As established chemical treatments for oilseed rape seed fall away, biological products have made a strong start as their likely successor. *CPM* tracks the story of Integral Pro, that marks this milestone achievement.

By Tom Allen-Stevens

It's a curious irony that some of the most brilliant innovations arrive virtually unnoticed but then dominate the market because their value goes almost without question. That may be the case with Integral Pro.

But this new seed treatment may herald the dawn of a new era: containing *Bacillus amyloliquefaciens* (BAA) it's the first biological seed treatment available widely in the UK for combinable crops and it has the potential to completely change the thinking on how growers nurture seedlings through those early stages.

“Integral Pro came out of the blue — few of us had heard anything about it until late in 2018,” notes David Leaper of Agrii. “But it slots perfectly into the hole in the seed-dressing market left following the withdrawal of thiram. It's now the market standard for oilseed rape.”

Very few biological products have ever stood up to the rigours of the four-point testing procedure Frontier puts new seed

treatments through, points out Jim Knight, the company's seed business development manager. “Integral Pro met the standard on all four key points, and that's fantastic — it sets a good example for what we can expect to see from biologicals going forward.”

Fungicidal effects

Integral Pro contains a bacteria, *B. amyloliquefaciens*, that's been shown to have fungicidal effects against soil pathogenic fungi (apart from *Pythiaceae*), responsible for damping off. It was introduced onto the market last autumn as a seed treatment for oilseed rape. What's new with this form of treatment, though, is that it stimulates the plant's own natural defences — you not only get improved plant establishment but it brings improved control on diseases such as phoma and alternaria and better vigour, notes BASF UK market manager for seeds Sarah Middleton.

“The effects of Integral Pro are proven — we know the mode of action (see panel on p78) and it translates into a yield benefit. What makes it different is that this is a biological treatment, so the extent of its effects depends on biological factors,” she says.

“The effects of biological treatments cannot fully compare to a chemical effect, but they are there and they are consistent. There's an acceptance with growers that this is part of an integrated approach to pathogen management which reflects BASF's focus on delivering sustainable solutions.”

But it's taken almost 40 years to get here. The initial discovery was made by scientists at the University of Nottingham. “We were

investigating the potential for biological control, testing a number of agents that were active and exhibited properties that could be fungicidal,” recalls Dr Steve Rossall.

“It was in 1981 we first noticed the properties of BAA. We found that botrytis species weren't developing on some leaf surfaces we were studying, indicating there were some antagonistic microflora at work. When we washed off the leaves and analysed what we had, that's when we found the bacteria.”

Another student at the university was carrying out a PhD study at the time, looking at the action of rhizoctonia on the roots of cotton plants. “We noticed when the cotton seed was coated in BAA, the bacteria colonised the roots and this had the effect of stimulating root growth,” continues Steve.

The information was passed to the Agricultural Genetics Company (AGC), which had been set up to commercialise the discoveries of what was then the UK



Integral Pro forms part of an integrated approach to pathogen management which reflects BASF's focus on delivering sustainable solutions, says Sarah Middleton.

Agricultural and Food Research Council. AGC was perhaps better known for its search for potential slug control agents that resulted in Nemaslug, a nematode now used widely by horticultural businesses and amateur gardeners. Along with its nematode projects, BAA passed to AGC's commercial arm MicroBio, purchased by Becker Underwood in 2000, which was subsequently acquired by BASF for just over \$1 billion in 2012.

Throughout this time, development work continued at the company's various UK-based sites, but the challenges were substantial. "It took us several years to get to a product that was ready to go to market," notes Dr Pamela Calvo Velez, BASF global fungicide and biological lead for seed treatment R&D.

"The first challenge is to scale up production. You're dealing with a living organism so first of all we have to create an environment and a process that

will encourage the bacteria to multiply. Then we have to scale up that fermentation process — it's one thing producing bacteria in a lab, but it can be very difficult to transfer this process to a large fermentation vessel," she explains.

This is a process that's been developed quite successfully at BASF's facility at Littlehampton W Sussex. It's claimed to be the largest 'farm' in the world, in terms of its throughput of living creatures, producing trillions of micro-organisms and nematodes every year.

Shelf life

The next stage was to develop a formulation, and this is where the team came across multiple challenges. "Shelf life is an important one," continues Pamela. "The bacteria must remain alive and effective in the final product for at least 18 months. Then, when it's applied to the seed, it must coat it correctly and remain viable on the seed for a period of months.

The seed must not be affected by the treatment, so we do a lot of monitoring work to ensure this.

"A crucial element with all biological material is that it is compatible with other products applied to the seed. We spent many years refining the formulation carrying out all the necessary checks before we launched BAA."

The final aspect, but arguably the most important was regulatory alignment. "This 7is where the BASF expertise really comes into its own," says Pamela. "We wanted a product that was compliant with European regulations first and foremost as these are the toughest globally. We have the processes within BASF that ensure every step of the development of a product meets those requirements."


The stakes were high — a delay in regulatory approval proves frustrating for the farmer and costly for BASF, she points out. "It's especially important with biological products that we get it



The bacteria need an environment and a process that will encourage them to multiply and it can be difficult to transfer this to a large fermentation vessel.

right — our focus in BASF is to bring on more sustainable solutions and biological products can help growers develop integrated crop management systems on farm. It's why we're keen to work closely with growers and develop knowledge in this area together."



The many years of painstaking research paid off — having finally developed the right formulation, the development team found BAA was a stable and robust strain. Applied at a rate of 160ml per 100kg, that puts 10,000 ▶



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Integral Pro has become the market standard for oilseed rape, says David Leaper.

► colony-forming units on each seed. These are compatible with a large range of existing treatments, including Lumiposa (cyantraniliprole), Cruiser (thiamethoxam), thiram and many of the biostimulant products used. Unopened, a pack lasts two years when kept at room temperature and the bacteria survives on the seed for at least 18 months.

BAA received its EU approval for use late in 2018 and Integral Pro was launched in time for the OSR crop drilled last autumn. But while the fungicidal properties

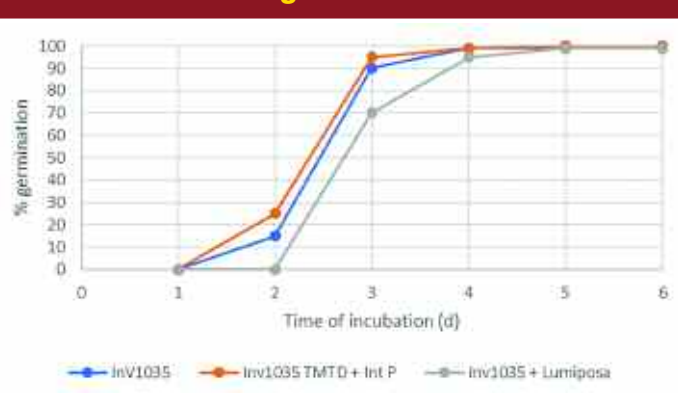
were clearly useful, it was the effect on plant vigour BASF was keen to explore further. Almost four decades after BAA left his lab, Integral Pro returned to Steve Rossall who put these claimed properties to the test.

“We tested four InVigor OSR varieties that were either untreated, treated with Lumiposa or with TMTD + Integral Pro. Each variety was replicated 10 times and tested at three different temperatures – 7°C, 10°C and 15°C,” he reports. “What we found was an increase in early growth for the TMTD + Integral Pro treatment, especially for the roots.” (see charts on right)

This vigour is significant, notes Sarah, and confirmed findings of field trials carried out across Europe. “One of the arguments against treating seed is that it slows down germination, and that’s certainly not the case with Integral Pro,” she says.

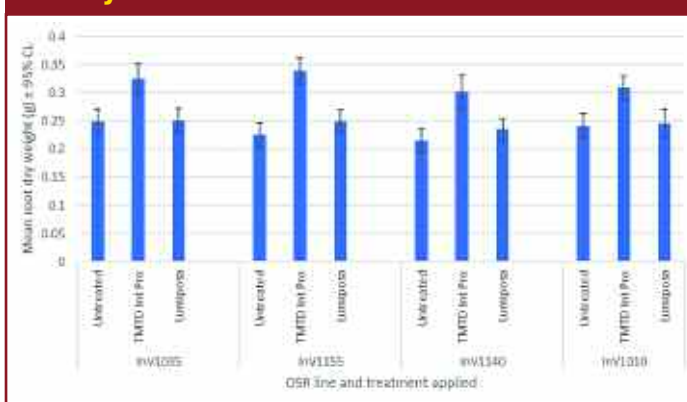
BAA has also been found to outcompete other seed-borne microbes, such as *Phoma* sp. and *Alternaria* sp., resulting in reductions in disease severity of 35% and 89% respectively,

OSR line InV1035 germination at 10°C



Source: University of Nottingham, 2019.

Effect of seed treatment on early root growth in OSR hybrid lines



Source: University of Nottingham, 2019.

How does Integral Pro work?

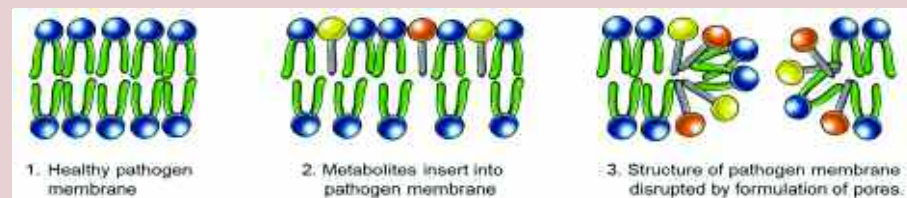
Bacillus amyloliquefaciens has three modes of action:

1. Root colonisation – a barrier against fungi

The bacteria develops a biofilm over the roots as they develop. They attach to the root surface as an extracellular matrix and then sporulate to spread further over the root network. This presents a physical barrier for fungal spores from entering the plant.

2. Anti-fungal activity via metabolite products

BAA produces iturin and surfactin (the yellow and orange metabolites illustrated below). These compounds protect the bacteria and secure resources from the environment to allow further growth. The metabolites insert themselves into the pathogen membrane, disrupting its structure and disturbing cell functionality.



3. Plant defence stimulation – Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR)

BAA stimulates both plant defences throughout its shoots, roots and leaves. SAR is induced by pathogens above or below ground and leads to the production of the phytohormone salicylic acid that activates pathogenesis-related genes. These produce proteins that thicken plant tissue, reducing its digestibility, and improving leaf growth. ISR is induced by certain non-pathogenic micro-organisms or elicitors below ground. The plant produces volatile organic compounds and proteins with anti-microbial properties.



The bacteria develops a biofilm over the roots as they develop which presents a physical barrier for fungal spores from entering the plant.



The production of antifungal compounds causes a zone of exclusion, where disease cannot grow and disease spores cannot germinate.

according to proprietary trials. "This is in line with standard seed treatments no longer available. But we've also seen some interesting effects with verticillium wilt. It's not completely controlling the disease, but shows a useful reduction in line with what you'd expect from a tolerant variety," notes Sarah.

Trials in mainland Europe have also shown reduction of leaf damage and larval burden of cabbage stem flea beetle by around 30%, although this effect was not seen under the high CSFB pressure we commonly see in the UK, she stresses. "Overall trials are showing Integral Pro delivers a yield advantage in the region of 0.16-0.18t/ha."

Key criteria

Any new seed treatment taken on by Frontier has to meet four key criteria, says Jim Knight. "It must be easy to apply by either a large commercial plant or a mobile unit. It should have a shelf life on the seed of at least a few months, and preferably allow for over-year seed. We need a shelf life in the can of at least 18 months, which gives us the faith to buy sufficient supplies when we may not know how much seed we're treating. And it must be compatible with other products.

"We've tested a lot of biological and biostimulant material and very few have stood up to the rigour of what we require. But Integral Pro does, which is very encouraging as it ties in with a more holistic approach to protecting a young

OSR crop at establishment," he says.

As a result, Frontier will be supplying seed dressed with Integral Pro as standard, with other nutritional and phosphite treatments to encourage early growth, and Polyseia, a polymer coating, specially formulated not to inhibit germination in dry conditions.

"What impresses us most with Integral Pro, though, are the results we've seen in field trials. At our Bleasby Site, near Friesthorpe, Lincs, it was the highest scored treatment for plant vigour, assessed 3 Oct 2019, while at harvest 2019 it provided an extra 200kg/ha in yield over a standard fungicide treatment, and 230kg/ha over re-cleaned seed."

David Leaper is also impressed by what he's seen in trials. "We've tested a lot of biological control agents at Agrii's Throws Farm site. On the whole, the control you get is variable. Integral Pro, however, has the same fungicidal effect as thiram, but appears to deliver a whole lot more."

Trials carried out at Agrii's northern sites under moderate CSFB pressure have backed up the BASF findings, he says. "One thing we've been watching for is anything that will bring about a delay in emergence. All the data shows that Integral Pro actually improves germination."

So Agrii is now rolling it out as a standard dressing across its OSR seed this autumn with phosphite-based Take Off as an option to improve establishment. "Some growers might consider going with undressed seed now that thiram is no longer available. We'd strongly advise against that as it opens up a whole host of risks for a crop that will likely face a lot of challenges. Integral Pro is a timely introduction — it's a biological, which must be borne in mind, but we see no negatives in using it, while there are a lot of positives to explore." ■

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