Working with biology's natural rhythm

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PROFESSOR GARY BENDING

A deeper understanding of what happens around crop roots – and crucially, when it happens – could eventually offer new ways to improve nutrient efficiency, manage disease and make better use of soil biology. *CPM* visits the University of Warwick Crop Centre to find out more.

By Charlotte Cunningham

The challenges facing crop production may look very different today than they did 75 years ago, but at the University of Warwick Crop Centre, there's a certain familiarity to the questions researchers are attempting to answer.

Based at the university's Wellesbourne campus, the centre traces its origins back to 1949 when the site was established as the National Vegetable Research Station in response to post-war concerns around food security and domestic production.

Today, climate change, geopolitical uncertainty, rising production costs and pressure on natural resources have once again put the resilience of UK food production firmly under the spotlight, says Professor Miriam Gifford, former head of Warwick's School of Life Sciences. "We can't just grow our way out of these problems. We need to be able to research and develop new varieties of crops and new ways of growing them which can help us."

PLANT-MICROBE INTERACTIONS

Central to that is the site's combination of experimental farmland and laboratory capability, allowing researchers to study crops under field conditions while also unpicking the biological mechanisms influencing their performance.

And among the relationships being investigated is one which takes place quite literally beneath growers' feet. Professor Gary Bending has spent much of his career studying plant-microbe interactions, with a particular focus on the rhizosphere – the interface between a plant's roots and the surrounding soil. "It's where you have water uptake by the plant, nutrient uptake by the plant, and also a huge microbial population," he explains.

In fact, much of the active microbial population in soil is concentrated immediately around the root, largely because plants themselves create the conditions for it to thrive, he continues. "Through photosynthesis, plants fix carbon which can then be released from their roots as compounds including soluble sugars and amino acids. Somewhere in the region of 5-8% of the carbon fixed by a plant can be exuded into the root zone."

Scaled across a crop or wider ecosystem, Gary says that represents a considerable flow of carbon directly into soil – and effectively provides food for the microorganisms inhabiting the rhizosphere.

Some of those organisms remain around the root, whereas endophytes can enter and live within the root itself. Others form familiar beneficial associations with plants, including the root nodule bacteria responsible for nitrogen fixation in legumes and mycorrhizal fungi involved in plant nutrition. But the microbiome also contains organisms which can negatively affect crop performance, making understanding its composition – and what shapes it – particularly important.

One of Gary's previous projects investigated exactly that in oilseed rape, examining how the crop's rhizosphere microbiome varies across space and time and whether those changes could help explain the yield decline associated with growing a crop too frequently. "Soils have different microbes and the environment can affect them – how cold or warm it is, whether it's dry, soil type, water availability and nutrient availability. Then the plant host itself has an effect; wheat will have a different microbiome to OSR, for example."

Researchers undertook landscape-scale sampling across England, profiling fungi, bacteria and protists within the crop microbiome while accounting for factors including soil, environment, cultivar and rotation. Importantly, farm records also allowed the researchers to connect those microbial communities with crop yield.

One organism in particular emerged from the work – the fungus *Olpidium brassicae*. "The shorter the rotations, the more you had a reduced yield compared with one-in-four, one-in-five or one-in-six rotations, and we found it was the abundance of *Olpidium brassicae* which affected landscape yield of OSR," says Gary.

The research also found that other pathogens could still be important at an individual farm level, but their relationship with yield was localised rather than apparent across the wider landscape, he adds.

At the other end of the spectrum, higher crop yields were associated with *Tetracladium* spp fungi., highlighting the potential importance of both beneficial and detrimental members of the microbiome.

For growers, rotation provides an obvious example of how that knowledge could influence management, believes Gary. "You potentially have beneficial microbes and potentially negative, deleterious microbes, and then you have the power to do something about it.

"You can look at genotype – can you select a genotype to recruit more beneficials or fewer deleterious microbes? Can you alter management practice? Rotation is an obvious one. If you know shorter rotations are going to result in more *Olpidium*, you can be more careful about rotation frequency."

Proven in the lab and the field

The University of Warwick Crop Centre, based at the Wellesbourne campus, combines experimental farmland and laboratory capability, allowing researchers to study crops under field conditions while also unpicking the biological mechanisms influencing their performance. *Photo: University of Warwick*

Resilient food production

Climate change, geopolitical uncertainty, rising production costs and pressure on natural resources have once again put the resilience of UK food production firmly under the spotlight, says Professor Miriam Gifford, former head of Warwick's School of Life Sciences. *Photo: George Archer*

AMPLICON SEQUENCING

The ability to investigate microbial communities at this level has been transformed by advances in DNA sequencing. Gary's earlier work used amplicon sequencing, which effectively produces a fingerprint of the microbial community. Researchers amplify a particular marker gene across groups such as bacteria or fungi, with differences in DNA sequences allowing individual organisms to be assigned a taxonomy. The result was the ability to characterise incredibly diverse communities which, just 20 years ago, would have been impossible to examine in comparable detail, he says.

But having developed better tools to understand which microbes are present, Gary and colleagues have now turned their attention to another question – *when* are they present and active?

The premise stems from the fact that plants, like humans, have a circadian clock or rhythm, says Gary – adding that somewhere around 30% of plant genes are regulated by that clock, allowing the plant to anticipate and respond to regular changes between day and night.

Crucially, the supply of carbon into the rhizosphere also appears to follow this daily cycle. "We know plants fix carbon in the daytime, and there's evidence that exudation largely takes place during the day. So you think, okay, that's feeding the microbes in the root zone. How are those microbes responding over daily timeframes?"

To find out, the team grew plants under controlled light-dark cycles and sampled their roots every three hours for 72 hours. DNA was extracted and amplicon sequencing used to track changes within bacterial and fungal communities.

But simply finding differences between day and night wouldn't necessarily demonstrate a genuine circadian response, warns Gary. "A circadian rhythm should continue even when the environmental cue responsible for synchronising it is removed. Much as a human body clock continues to operate if someone is placed in constant darkness, a plant's internal clock can continue to run without the normal light-dark cycle."

Researchers therefore also used plants with an impaired circadian clock and moved plants into constant light conditions. The results showed that a proportion of both fungal and bacterial communities followed clearly defined cycles, increasing and decreasing in abundance over the course of the day. "Some were more abundant in the day and some were more abundant at night, but they showed true rhythms," explains Gary.

For most of the organisms displaying those patterns, rhythmicity continued under constant light, indicating a connection with the plant's circadian processes rather than microbes simply responding directly to daylight. "Around 10% of the microbial community displayed these rhythms. So we know the rhizosphere is chemically rhythmic because exudation happens largely in the day and you don't have those soluble sugars and soluble carbon available as

A career below the ground

Professor Gary Bending has spent much of his career studying plant-microbe interactions, with a particular focus on the rhizosphere – the interface between a plant's roots and the surrounding soil.
Credit: George Archer

much at night. But we now know it's also a microbially rhythmic environment."

And it isn't simply microbial abundance which changes. Among organisms favoured during the daytime, Gary says a relatively large proportion were anaerobic or facultatively anaerobic, whereas organisms becoming more abundant at night tended to be aerobic.

He believes that provides another clue about what's happening within the root zone. "During daylight, plants supply carbon to the rhizosphere. Microorganisms utilise that carbon and, in doing so, consume oxygen, meaning the environment immediately surrounding the root can become increasingly oxygen-depleted."

This becomes particularly interesting when viewed alongside nitrogen cycling, he adds. "Nitrous oxide emissions from agricultural and other ecosystems have been shown to display daily rhythms, while plants themselves don't take up nitrate and phosphate uniformly throughout the entire day and night."

Together, Gary believes it raises the possibility of a chain of interconnected processes – the plant's circadian clock influencing carbon exudation, which drives microbial activity and oxygen consumption, which in turn affects microbial processes involved in nitrogen transformations.

It's these relationships which Gary's latest research, supported through a European Research Council Advanced Grant, will investigate in greater depth. "The idea is to redefine the rhizosphere as a rhythmic compartment within the soil," he says.

Carbon supply is one possible driver, but it isn't the only one, he adds. "Plant immunity also changes over diurnal timeframes. Defence pathways involving salicylic acid and jasmonic acid fluctuate through the day, and says there's evidence that some pathogens can exploit periods when plants are more vulnerable because of the crossover between these immune responses."

Consequently, changes in plant immunity could be another factor shaping which organisms colonise the rhizosphere – and when, he adds.

The project will also investigate what causes microbial populations to decline after they've proliferated. "If you give a microbe carbon in the daytime it'll grow, so you can understand why you might get increased growth during the day. But what's driving the turnover? What's driving the reduced abundance at night? And equally, what about microbes which are more abundant at night and decline during the daytime?"

> ***"The idea is to redefine the rhizosphere as a rhythmic compartment within the soil."***

One possibility involves bacteriophages – viruses which infect bacteria. Gary points to marine ecosystems, where phages are responsible for turning over a substantial proportion of cyanobacterial populations on a daily basis, creating a microbial loop which operates over diurnal timeframes.

As such, he says the research will investigate whether comparable phage-driven turnover occurs within the rhizosphere.

At the same time, sequencing capability is taking another considerable step forward, adds Gary. Rather than examining only individual marker genes, the new research will use long-read sequencing technology to assemble whole microbial genomes.

This means researchers can investigate the genetic repertoire of organisms showing rhythmic behaviour, as well as studying gene expression to establish which genes are actually active at different points through the day. "In simple terms, the science is moving from identifying who is there, towards understanding what they're capable of doing – and when they're doing it."

Barley will be used within the project, with research moving beyond controlled laboratory systems into the field to investigate how strongly these biological rhythms persist alongside changing temperature, sunlight and soil moisture.

As with all research, proving it in a laboratory is one thing, but making it work commercially can be entirely another. So where could that eventually take crop production?

Gary is clear that understanding the mechanisms comes first, but the potential applications are significant. "If you understand how the plant is directing the soil around it and the microbes around it, probably to sustain its own nutrition, then potentially you can manage that.

"You might be able to manage it through plant genotype – selecting plants with particular traits which allow them to harness those microbes better, or time the processes going on in the rhizosphere better to reduce fertiliser applications and increase fertiliser efficiency."

Timing inputs could eventually provide another opportunity. "If particular pathogens become active at predictable points in a daily cycle – or plants themselves become more susceptible at certain times – crop protection applications could potentially be timed to coincide with those periods."

Likewise, understanding when plants are taking up nutrients and when microbial communities are driving nitrogen transformations could help inform strategies to improve fertiliser-use efficiency and reduce losses, he says. "It's an emerging area of chronobiology – considering when biological processes occur rather than simply whether they occur."

There could even be opportunities for plant breeding, and Gary says research with plant circadian biologists suggests some of the rhythmic characteristics found in wild populations have been lost from modern crop plants during breeding.

Whether future genetics could make better use of those characteristics – potentially producing crops better able to synchronise their biology with beneficial microbes, nutrient availability or environmental conditions – remains to be established. But for Gary, that's precisely why understanding the mechanisms taking place within the rhizosphere now matters... ●