

The molecular momentum of pulse progress

“This can do super-exciting real-time diagnostics.”

Innovation Genetics

Cutting-edge technology in genomics and bioinformatics is being harnessed to improve the resilience and uses of pulse crops. *CPM* takes a tour of the labs at Norwich Research Park.

By Tom Allen-Stevens

What he holds in the palm of his hand can do in a matter of hours the DNA sequencing that once took decades. It's the size of a mobile phone but is capable of unravelling an entire genome — work that less than 20 years ago was done by teams of top scientists and the world's most powerful computers at a cost of more than £1bn. But the Nanopore MinION you can buy on the internet for just \$1000 (£714).

“This can do super-exciting real-time diagnostics,” enthuses Peter Bickerton of the Earlham Institute within the Norwich Research Park. “You can take it to a rain forest, to a marine environment, to outer space or to an arable field. You just plug it

into a laptop and it gives you real-time species ID or analyses the DNA of pathogens, foodstuffs, or any other organism.”

In the same room are dozens of sequencing machines which are picking their way through the DNA of anything from an aphid, to a cow, to Chinese spring wheat. The PacBio RS for example — still at the cutting edge of DNA sequencing, but the size of a small car, so now looking a little cumbersome — is busy sequencing the genome of a worm found in a volcano.

Decoding living systems

“We apply high-performance computing resources to decode living systems,” explains Peter. “We can sequence single cells and look at how biosystems interact. Only about 2% of DNA is believed to define life. But we also look into the 98% known as “junk” which might actually have a very important role.”

Samples come in from all over the world. They're prepared then fed into the machines that unravel their code. The Illumina HiSeq 4000, for example, looks in some detail at fragments of 700-800 base-pairs before these are stitched back together again, like a giant jigsaw puzzle,



Peter Bickerton with the HiSeq 4000 that takes about a week to accurately sequence a wheat genome.

to build an entire sequenced genome — wheat has a total of 17bn base-pairs.

The PacBio takes 30,000 base-pairs at a time. “It's less accurate, but analyses longer stretches, so tells you more about the structure. The MinION essentially does the same job but it's about 15% less accurate, and for long reads you really need the accuracy of a PacBio. It now takes just days to map an entire human genome, for example, and the information for all our reads is then made publicly available.” ▶

BOOST BLIGHT PROGRAMME WITH PREVENTATIVE PROXANIL®



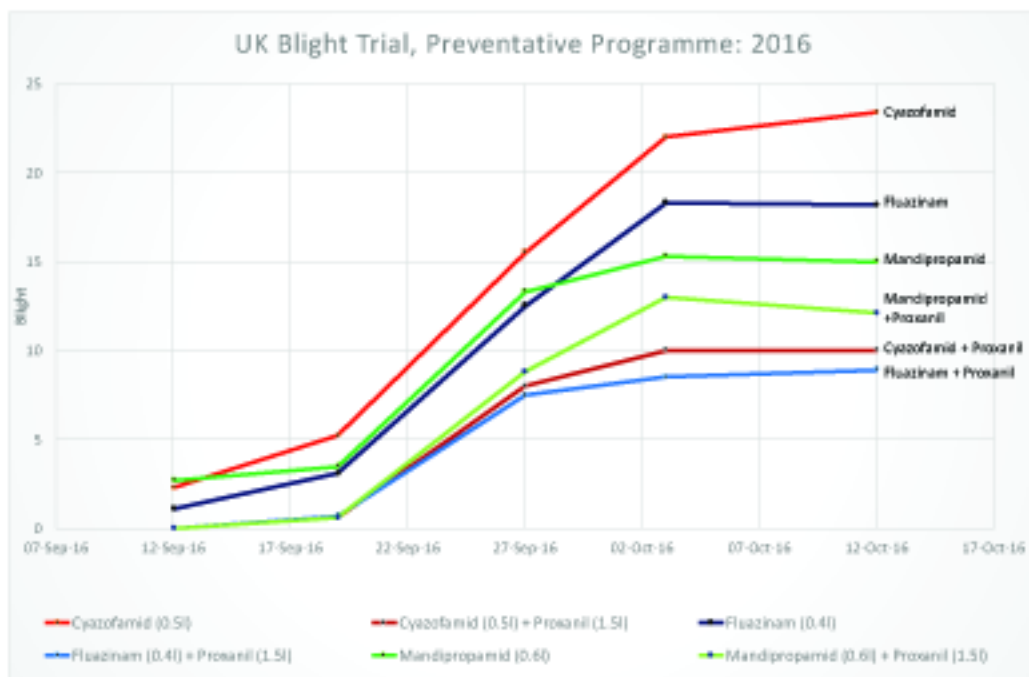
Using Proxanil® as a preventative product boosts the overall efficacy of blight control programmes, is the message for growers looking for an extra level of protection.

Proxanil, by Arysta LifeScience, combines the power of two complementary active ingredients – Cymoxanil and Propamocarb. When used in conjunction with a contact fungicide, trials have shown that Proxanil yields an additional benefit for the control of potato late blight.

Arysta LifeScience Product Development & Technical Manager for UK & Ireland, Don Pendergrast, said: "We have been collating trial data on this for the past four years. Results consistently show that using Proxanil, which has a curative effect, as part of a preventative blight programme, will improve overall efficacy.

"The trials, which took place in Lincolnshire, looked at the application of Proxanil alongside blight-controlling contact fungicides such as cyazofamid, mandipropamid and fluazinam.

"We are delighted with the



results that show Proxanil improves the efficacy of all these leading active ingredients, boosting their performance throughout the season."

Proxanil, a semi-systemic fungicide with translaminar effects, works by combining propamocarb with cymoxanil. Propamocarb penetrates the leaf tissue to prevent blight infections and ensure protection for new plant growth, whereas cymoxanil acts rapidly to provide a strong eradicant effect in the first 48hrs after application, and an ongoing control for around two further days.

The trial results show the benefit of selecting a variety of products for use in combination, a tactic which also reduces the risk of resistance.

Mr Pendergrast added: "Late

blight has a huge impact on the potato industry, costing around £50M in crop protection products each year. It's aggressive and once symptoms are visible, it has already taken hold.

"It's been a challenging time for potato growers thanks to the rising threat of fungicide resistant genes, particularly the increasing incidence of EU-37

(dark green) which is less sensitive to fluazinam.

"Using Proxanil in a preventative spray programme with a contact fungicide improves the robustness of a late blight programme."

For preventative use, Proxanil should be used from the rapid growth stage onwards, as part of a programmed approach.

About Arysta LifeScience

Arysta LifeScience is a global agricultural company specialising in the marketing and distribution of innovative crop protection and life science brands. Arysta LifeScience has a well-integrated biological and chemical portfolio to provide complete solutions to growers including biosolutions, fungicides, herbicides, insecticides and seed treatments.

For more information on Arysta LifeScience UK, visit www.arystalifescience.co.uk



DON PENDERGRAST
Arysta LifeScience Product Development & Technical Manager for UK & Ireland.

Using a variety of modes of action in a programmed approach is the best tactic for disease and resistance management.

► It's this sort of technology that's bringing scientists a much greater understanding of what genes do and how living systems interact. "You can use the information for gene-editing, for example. This is where you take a genome and cut bits out, re-order or insert bits," explains Peter. "It allows you to take disease resistance, for example, from one variety of the same species to another."

Scientists at the John Innes Centre next door are close to pinning down reliable disease resistance to downy mildew that can be bred into commercial pea and bean varieties.

Molecular markers have now been identified for two genes that specifically relate to disease resistance in peas, reports JIC's Prof Claire Domoney. "We've known of genetic resistance in one line,



Clare Domoney and her team have identified a second pea line that exhibits a hypersensitive reaction to downy mildew.

J1 15, for some time, but the industry has been reluctant to rely solely on it because of the potential for the pathogen to overcome resistance. Now we've identified a second line that exhibits a hypersensitive reaction to the disease."

The second source of resistance is a wild accession from Afghanistan. "It was overlooked in previous studies, possibly because it works by containing the pathogen, which limits how it affects the plant."

Downy mildew

The discovery is important because downy mildew is a devastating disease in both peas and beans. Control in peas in particular is limited to use of the seed treatment Wakil XL (cymoxanil+ fludioxonil+ metalaxyl). Following a recent regulatory change that came into full effect this year, only crops planted after 1 April can be treated.

A pre-breeding programme is now underway at JIC to explore the two-gene interaction — 195 F3 and F4 lines are being tested for downy mildew resistance and susceptibility. But even this won't be enough for commercial lines, notes Claire. "We need at least three genes for robust resistance, but we hope that finding further genes and molecular markers should be fairly straightforward given new technologies."

The work is part of the Pulse Crop Genetic Improvement Network (PCGIN), a major industrial stakeholder-linked project



The size of a small car, the PacBio is still at the cutting edge of DNA sequencing and takes 30,000 base-pairs at a time.



Real-time sequencing can be done in the field with the Nanopore MinION – the kit with disposable flow cells is available to buy on the internet for \$1000.

to which Defra has committed another five years of funds up to 2023. There are four work packages in the new programme:

Pulse quickens with public funding

Whether your pea is smooth or wrinkled could become a key consideration for those with type 2 diabetes. Scientists have discovered a link between the genetic trait and the type of starch the pea contains.

"Type of starch is important, and it relates to the amylose: amylopectin ratio," explains Claire Domoney. "Long unbranched starch chains take longer for the digestive enzymes to break them down. But if the starch has many short branches, this is broken down quickly which can cause problems for those with diabetes.

"The genetic link between the type of starch produced by a pulse crop and whether it is wrinkled or smooth is well-known. But there's a lot more variation in the starch branching within germplasm than we'd anticipated — we're just at the tip of the iceberg of discovery on this trait and its properties."

The work is part of the BBSRC Diet and Health Research Industry Club-funded CRESTAR project,

that is using crop genetics to understand the importance of dietary resistant starches for maintaining healthy glucose levels.

Likewise the PeaGen project is another five-year, BBSRC-funded programme involving an industry consortium that'll help develop new peas to replace soyabean in the diets of poultry and monogastric livestock.

"One problem being investigated is the negative relationship between protein and yield. We've developed mutant lines where we've knocked out blocks of seed protein genes. This has resulted in lines with a different protein composition, but so far no apparent gain in yield, at least in the laboratory lines and conditions," reports Claire.

But the pace on exploring these genetic traits and developing the germplasm has suddenly taken a big step forward thanks to new speed-breeding techniques. Claire explains it should address the problem of not having enough material to test an interesting new trait, for example.



New speed-breeding techniques have reduced the generation time to under 50 days and could bring farmers in on the voyage of discovery much earlier than before.

"Breeders will ask us for kgs of seed to test a new trait, but all we'll have is a handful. Now we reduce the generation time to under 50 days and breed up to eight generations in a year. This will dramatically accelerate research and breeding, and turn an interesting new trait into something of commercial value much faster. It'll also mean we can bring farmers in on the voyage of discovery much earlier than before," she notes.



Dr Noam Chayut is JIC's Germplasm Resource Unit manager, and looks after its 3200 diverse lines of pea.

- WP1: A platform that supports UK pulse crop improvement
- WP2: Genetic knowledge and resources for plant and seed traits in pea and faba bean crops
- WP3: Toolkits for legume crop-breeding programmes
- WP4: Opportunities for UK legume crop improvement through UK and European collaborations

"One of the really positive aspects about this project is the number of companies and institutions now involved," notes Claire. "We started with just five stakeholders, but the stakeholder listing now stands at 136, of which 85 are non-academics with good representation and active involvement in evidence at the last stakeholder meeting."

Unlike the wheat genome, the pea genome hasn't yet been sequenced, although this is expected to come sometime this year. "Without a genome sequence, we're heavily reliant on finding genetic markers by other means," explains Claire. It's these genetic markers breeders rely on to quickly identify progeny from crosses that exhibit traits such as disease resistance or quality criteria.

One notable success has been colour stability in peas — bleaching is a problem that can easily downgrade a premium crop, resulting in heavy financial losses for the grower. "There's a strong association between loss of colour and genetic markers we've identified," says Claire. "So we've been looking through the germplasm library to identify lines that retain their colour."

JIC houses the Germplasm Resources Unit that has gathered from around the world and now stores under controlled conditions a valuable collection of diverse lines of pea. That amounts to 3200 accessions, including wild, natural lines, landraces, modern cultivars and research lines, including mutant material.

Colour-stability trait

"We've identified six new sources of variation in one gene that controls the colour-stability trait," reports Claire. "This is largely within landraces and wild backgrounds, so the challenge is to use these types to move the gene into a common background."

And there's a new rapid testing technique JIC has developed that's helping here. Scientists have discovered they can infiltrate leaves of a plant with different genes or combinations of genes and study how the plant responds. The technique is so successful it's been spun off into a separate entity, Leaf Systems, that uses the technology to quickly produce proteins in plants such as vaccines, antibodies or enzymes.

"It's a very robust system for testing different genes for their function and we have some clear results of greener leaf material, compared with controls," she says.

But it's not just on the agronomy side where traits are being explored and pinpointed. This voyage of gene discovery is beginning to unlock feed and digestibility properties of pulse crops, which could enhance their value in commodity and premium markets.

"One of the problems of pulse crops as feed is that they have a protein digestion



There's huge potential to explore traits, especially through using mutants, such as this leafless pea variety.



Leaf Systems uses a new rapid testing technique that infiltrates leaves of a plant with different genes to assess how the plant responds.

inhibitor that prevents animals fully utilising the seed protein as a food source. Some GM soya lines lack that inhibitory activity, but there are no known natural sources in commercial pea and bean crops," explains Claire.

But a mutant pea line, *Pisum elatius*, has now been identified with unexpectedly low inhibitory activity. What's more, scientists have pinpointed the two genes responsible, which have lost their function in the mutant. "The question now is whether these genes have functions beyond protein inhibition, such as a positive effect on yield or resistance to pathogen or insect attack. Initial studies suggest not, but it's early days."

Similar work has been investigating the functionality of pea flour. "It's a valuable source of wheat-free food products, for example for coeliacs. But dough made with pea flour doesn't rise as well as that of wheat. We think we can change the composition of certain proteins to develop lines with different performance," says Claire.

As discoveries are made, these are fed through to commercial breeders in the form of "toolkits" — a set of genetic markers and germplasm that will allow them to bring the traits into commercial lines. The aim is to accelerate the discovery and commercial adoption of high performance lines with improved functionality and reduced reliance on pesticides.

"There are some perfect genetic markers, that have a direct connection with a phenotypic trait, such as the round or wrinkled genotype in peas. Others are imperfect — we've identified genes either side of the trait gene, such as for downy mildew. But all should help breeders make step changes in the varieties they develop, and we're learning a lot as we go about the techniques and methods that'll unlock this material's genetic secrets," concludes Claire. ■