Crawford Fund FOR A FOOD SECURE WORLD

CELEBRATING AGRICULTURE FOR DEVELOPMENT

Outcomes, impacts and the way ahead

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Editor **Ann Milligan**

RNA-based biopesticides for sustainable agriculture: BioClay™ technology

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This talk is about RNA-based biopesticides for sustainable crop protection: the BioClay™ platform innovation, which is a specific innovation with a broader impact. We started working on RNA sprays for crop protection about ten years ago – an innovation that may have been an agricultural genesis for today's RNA-based vaccines for COVID-19.

We have been reminded – both by Dr Audrey Aumua yesterday, and again by today's speakers – about the significance of plant health for food and nutritional security. We do need pesticides to protect our plants from crop losses. However, there are issues, such as resistance, residues, runoff to our precious waterways and the significance of the blue–green economy, and also the issue of lack of specificity. We do not want to harm our beautiful butterflies and bees, yet new chemicals are hard to find. There are now also global imperatives, with the European Green Deal in 2020 setting a target of 50% reduction in pesticides by 2030.

Within that landscape, we need innovative tools to grow safe food. I am not saying we can find a silver bullet. However, we can look for integrated pest management strategies, and develop tools that resonate with sustainability and that show some responsibility towards the planet.

For me personally, a trigger to working on RNA-based crop sprays was the news in 2013 that 25 children in India died after consuming a community meal that had toxic pesticides. Things like that do happen, and we need to look for solutions.

When I started work on RNA sprays, there were already people working on RNA sprays for crop plants. However, there were problems: RNA is a very fragile molecule, highly unstable, and it gets washed off by rain, so the protection window was lasting maybe only 3 to 5 days. The challenge we took on was how to convert these RNA sprays into a system that is commercially viable for farmers, that is environmentally friendly and non-toxic to humans, is stable, sticks to the leaves but has no residue, is not washed off by rain, is easy to adopt, and protects for more than 3 to 5 days. In other words, a system that can be translated for adoption, and which can make a difference on the ground – all features that will make it more viable. And that is where the concept of BioClay was born. 'Bio' in BioClay™ is RNA, which is the biologically active ingredient, and the clay particles are the carriers of the RNA, in a way that provides the spray with all those advantages and features that we were looking for.

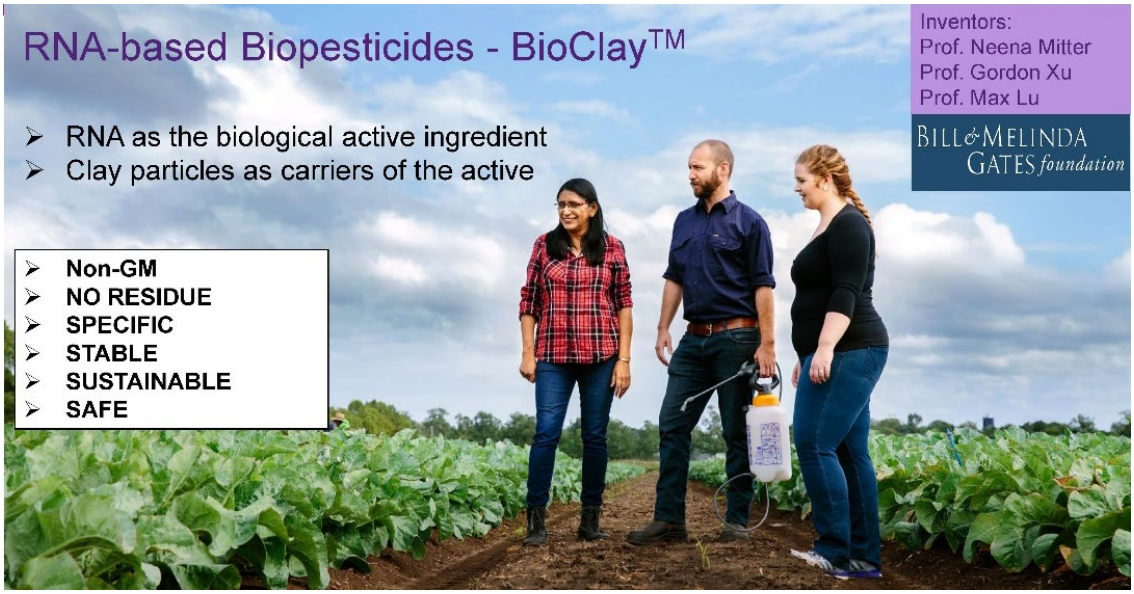

Figure 1. Prof Neena Mitter, Dr Karl Robinson and Dr Elizabeth Worrall.
Photo: The University of Queensland ©

This idea was first funded by the Bill and Melinda Gates Foundation (Figure 1). Later on, Nufarm Australia Ltd joined as an industry partner and we received funding from the Queensland Government followed by other multiple grants from Rural Development Corporations and the Australian Research Council. The team has published in *Nature Plants*, and the young leaders have gone from strength to strength, which resonates with the theme of this conference – that we need to support the flagbearers of tomorrow.

BioClay™ and progress so far

The clay, as I mentioned, is just an inert clay: it is just magnesium and iron, and the RNA is the biological active. The clay particles are positively charged; the RNA is negatively charged. You mix the two and you get BioClay™. The spray does not alter the plant genome, and the clay layers degrade naturally, leaving no residue. BioClay is stable enough for slow release of the dsRNA on the plant surface. The important point is that the RNA in this comes from the pest or pathogen itself, to kill the pest or pathogen. It is almost 'nature versus nature'. I will not go into the details of the science of the RNA interference technology.

Here is a brief snapshot of the progress we have made with BioClay over the years. We started working with vegetable crops. Figure 2 shows capsicum plants sprayed and unsprayed. It works! This was our first system, and we were very excited.

We then moved to other viruses. Zucchini yellow mosaic virus (Figure 2) was a learning curve for us when we transitioned from the lab to the field. I still remember the phone call from my post-doc saying, 'Neena, do you know how big zucchini grows in ten days in the field? They are really huge plants. I never thought it would grow like that. We need to optimise our dose and spray regimes.'

That experience highlighted that it is very important to bridge that gap between lab to field – and the engagement with growers and the engagement with industry partners – right up front, to have success.

Figure 2. It works! *Top:* Tomato spotted wilt virus in Capsicum. *Below:* Zucchini yellow mosaic virus.

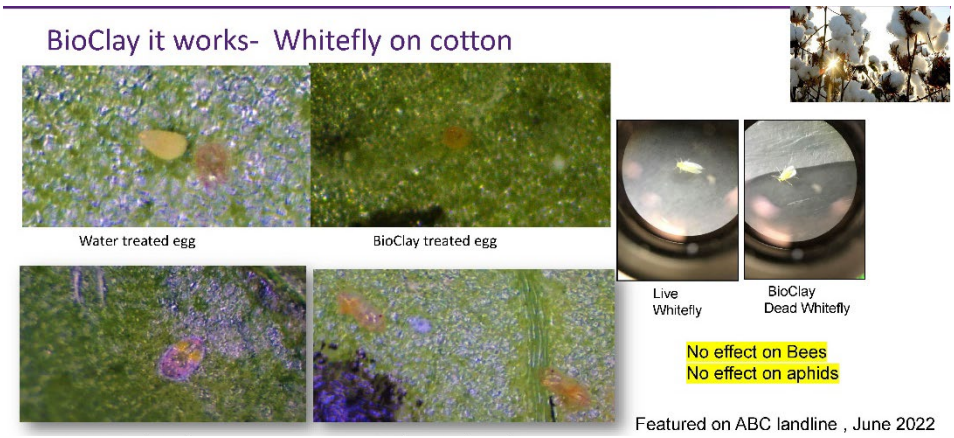

Figure 3. BioClay™ targeting silver leaf whitefly

We have now added work on insect pests, such as whiteflies on cotton (Figure 3). We find that BioClay can target not only the adult whiteflies but also multiple stages of the lifecycle of whitefly. This was research by a PhD student who was a Crawford Fund scholar in 2019, and he also has published in *Nature Plants*, and been named as inventor on patents, and the work was featured on ABC *Landline* in June 2022. This is another example of how important it is that we inspire young researchers to take up these innovative concepts, work through all the problems and challenges, and succeed ready to take on new challenges.

Figure 4.

We are also looking at fungi because fungal diseases are highly significant. We have an Australian Research Council Research Hub, and our team of three who started the work on BioClay is now a team of 50! (Figure 4). It is through collaboration, through partnerships, that the BioClay work has progressed the way it has, in partnership with Nufarm Australia Limited and multiple Rural Development Corporations such as Horticulture Innovation Australia, GRDC, CRDC, Wine Australia, and other academic and government partners across Australia and internationally. It also brings support from the Department of Agriculture and Fisheries, Queensland. For fungi, we are working on some of the key crops, based on industry feedback: *Sclerotinia* in canola, *Verticillium* in cotton, *Botrytis* in grapes, chickpea and strawberries, and

Figure 5. Chickpea

Fusarium in wheat. I can tell you (this is 'hot off the press'!) that we are getting early success with *Botrytis* on mature chickpea plants, where once again BioClay provides an extended window of protection (Figure 5).

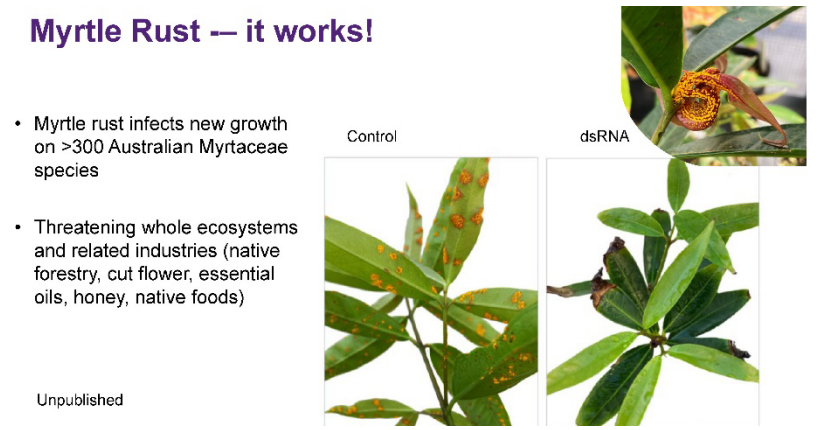

Figure 6.

This has also inspired some of the young girls in our team specifically to look at BioClay for tree crops. One of the senior postdoc researchers and her PhD student are looking at myrtle rust – a very significant issue for multiple Myrtaceae species, and we are getting some very nice early success (Figure 6), though there is still a long way to go.

The important part is that when we design this process, we believe in co-designing and co-creating it with industry partners. Our ARC Hub (Figure 7) does not just talk about science; it talks also about scaling-up as one of the themes for RNA and clay manufacturing; and it takes into account the registration and the regulatory components of the work. Most significantly, we have a team in the Hub to consider social licensing. An entire project team focuses on how we will engage with the growers; how we will engage with the consumers; what will be the adoption issues. That becomes part of this Hub's translational process as well.

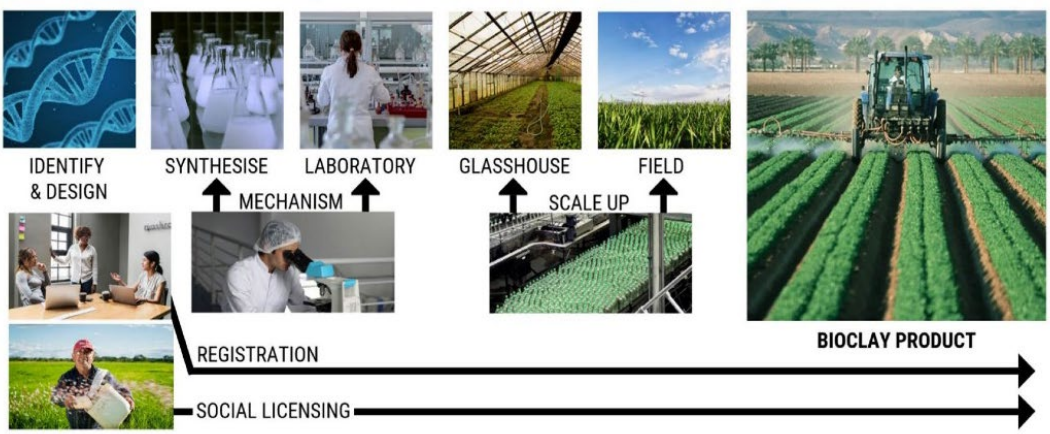

Figure 7. The Research Hub process.

On 8th October 2019 the Australian Parliament formally agreed with the Office of the Gene Technology Regulator's proposal that topically-applied dsRNA be exempt from GMO regulations

Gene Technology Amendment (2019 Measures No. 1) Regulations 2019

24 Schedule 1A (at the end of the table)

Add:

11	Introduction of RNA into an organism, if: (a) the RNA cannot be translated into a polypeptide; and (b) the introduction of the RNA cannot result in an alteration of the organism's genome sequence; and (c) the introduction of the RNA cannot give rise to an infectious agent.

Figure 8.

In relation to GM regulation and policy, the Office of the Gene Technology Regulator has now passed legislation, that topical application of RNAi is non-GM (Figure 8). That is a big step. It means BioClay™ does not require any deregulation here. It is also the case in USA. We hope that Europe may decide similarly, sometime in the future.

For trade and markets, the decision is helpful, as it means short or nil withholding periods for produce for sale in domestic markets, and short or nil intervals before export (to satisfy standards imposed by overseas trading partners). It gives BioClay™ treatment the possibility to be used more widely, which has implications for value chains, and food security, and the future consumer as well (Figure 9), because the consumer of today is very conscious about provenance, looking at the credentials of food production. That will affect the cost-effectiveness, and the wider reach of the BioClay™ platform as well.

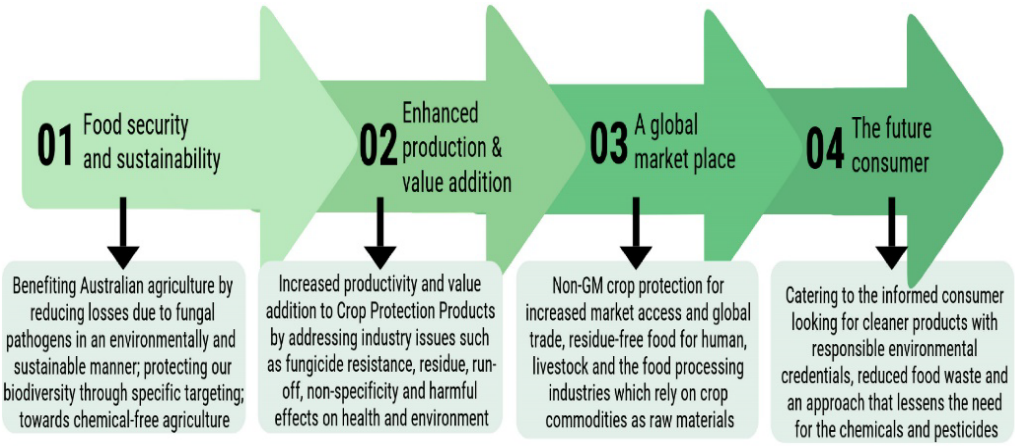

Figure 9.

We are looking at biosecurity issues. We are even investigating animal health with BioClay™ treatment – it appears to have a wider reach, which we can tailor (Figure 10). It may not work for everything, but at least we can try a range of applications and see which ones work. The key thing is that regulation, policy and public opinion are crucial in this area of research. There is still a way to go to develop BioClay as a product and make it a reality.

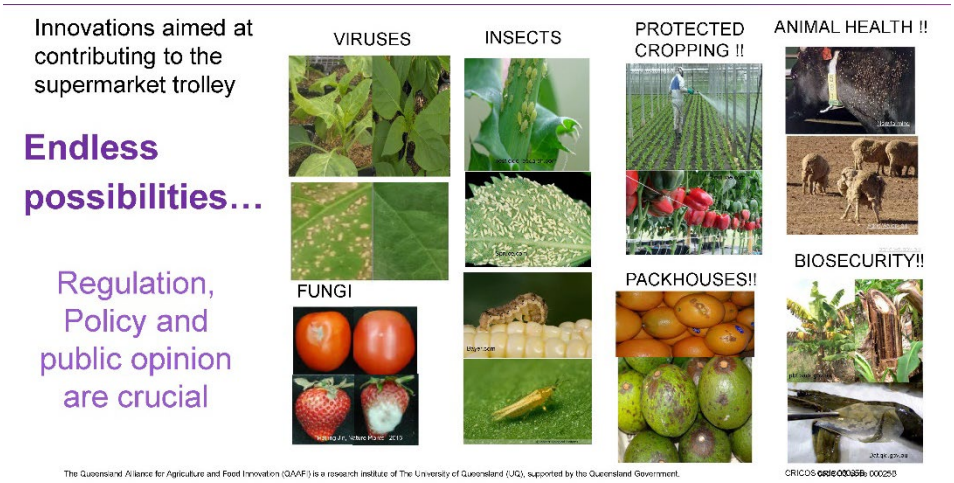

Figure 10.

Acknowledgements

Thanks to everyone who has now worked with us on the BioClay team. It takes a village to grow those innovations, and I am very proud that we have built that village.

Professor Neena Mitter is the Director of the Centre for Horticultural Science at QAAFI (Queensland Alliance of Agriculture and Food Innovation), at The University of Queensland (UQ), and the Director of the Australian Research Council Industrial Transformation Research Hub for Sustainable Crop Protection. Her career and passion for delivering real world outcomes have received recognitions such as Fellow of the Australian Academy of Technology and Engineering, 2021 Pravasi Fellow of the Indian National Academy of Agricultural Sciences, Australian Women in Technology Outstanding Life Sciences Award, and Gates Grand Challenges Explorations Award. Professor Mitter has over 120 publications and has supervised >20 PhD students. She is globally renowned for her leadership of innovative platforms: namely, 'Environmentally sustainable BioClay platform for crop protection' and 'Clonal propagation of avocado using plant stem cells'. These are ground-breaking platform technologies influencing agricultural production, environmental sustainability, and socio-economic dynamics of farming communities. Professor Mitter is also championing a UQ wide initiative on 'Protected Cropping for Tropics and Subtropics'. With increased scrutiny on use of chemicals as crop and animal disease control agents, Professor Mitter is focused is on developing clean technologies for the agriculture of tomorrow. As Chairperson of UQ Cultural Inclusion Council and Deputy Council member of the Australian Human Rights Commission Leadership Council on Cultural Diversity, Professor Mitter is at the forefront of diversity and inclusion initiatives.