

A collaborative project between CSIRO, Bayer and CRDC

Rapid monitoring of molecular resistance in *Helicoverpa*

■ By Dr Amanda Padovan – CSIRO Land and Water

WOULD you like to know what insect pests are on your crop right now? How about what they are resistant to? With this information you could build a dynamic crop protection plan tailored to your farm that incorporates resistance management. Developing a cost effective, user friendly and reliable molecular resistance monitoring tool is the first step to making this dream a reality.

The current bioassay test for resistance monitoring in *Helicoverpa spp.* is labour intensive and therefore expensive and time consuming. Only a relatively small number can be tested over the course of a season and management decisions based on resistance levels can only be implemented in the next growing season. High throughput molecular resistance monitoring could allow changes in resistance levels within fields to be detected which would enable active pest management by conventional means within growing seasons, using rapid data generation and large samples sizes.

Molecular resistance monitoring tools

A collaborative team is developing molecular tools to monitor resistance to *Bacillus thuringiensis* (Bt) insecticidal proteins and conventional pesticides in the cotton bollworm, *Helicoverpa armigera* and the Australian bollworm, *Helicoverpa punctigera* in Australian cotton fields. Bayer, CRDC and CSIRO recognised the potential benefits of molecular resistance monitoring and have co-invested in this proof-of-concept project. We see these tools ultimately being expanded to work with all agricultural pests and crops worldwide. We are developing three molecular tools for different applications.

Whole genome sequencing

By far the best tool, in terms of detecting resistance, involves sequencing the whole genome of each individual insect. This method replaces and improves significantly on the current bioassay tests but is relatively costly at \$20 per sample. The innovation is in how we analyse and use the data.

This method collects data on the whole genome of an individual which allows us to look for all resistance genes that are currently known and newly detected into the future and helps to generate a layer of data that is built on over time. The whole genome data of these insects will be stored in a database that can be queried at any time for any purpose.

We are already using existing data to assist the cotton industry. For example, we have a database of about 300 samples of *H. armigera* that we queried for the dominant Cry1Ac resistance allele found in China in 2018. Within a few hours we knew that this allele hadn't been detected in any Australian samples collected thus far. In addition to resistance monitoring, this data layer can be used to understand the population of insects present in cotton growing regions and relate it back to management: Where do the insects come from? Are the populations connected throughout the growing region, such that resistance can spread from one area to another, or are there distinct populations in each valley or farm? Are our current resistant management practices working as they should? Do moths from refuges move into fields of Bollgard 3 cotton? Do we have to make changes to the Resistance Management Plan (RMP) to reduce the likelihood of the evolution of resistance or, are we able to revisit the tactics we are using?



Amanda Padovan.

Target enrichment sequencing

We are developing a targeted sequencing approach which replaces and improves on the bioassay-based resistance monitoring. It costs less than the first approach, at around \$5 per sample, but also generates much less data.

The targeted sequencing approach detects all resistance genes currently known but cannot always detect new resistance genes as they are reported. The genes must be included in the design and if there is no evidence for a gene to be involved in resistance then it won't be included in the design. For example, the dominant Cry1Ac allele reported in China, would not have been found using a target enrichment method because there was no evidence that the gene (a tetraspanin gene) could be involved in resistance and so it would not have been included in the target enrichment design.

We have targeted the genes known to be involved in resistance and those highly expressed in the midgut surface cells that are affected by Bt toxins (i.e., genes that could be involved in resistance). The data from this tool can be used to build a dynamic resistance management plan and improve the efficiency and effectiveness of pest management in Australian cotton fields— that is, the data will more readily support changes in the RMP between seasons.

Direct Polymerase Chain Reaction (PCR)

As populations change and evolve over time, it would be necessary to continue to perform some of the current F2 bioassay tests each year to keep the databases generated by whole genome sequencing and target enrichment sequencing up-to-date. The final tool, a direct PCR method, allows researchers

to very quickly determine whether an insect that survives an F2 bioassay has a known resistance allele without needing to kill it to obtain molecular DNA. If the insect contains a new resistance allele, the researchers try to establish a colony from this unharmed specimen for further characterisation. Due to costs and limited scalability, we don't advise that this tool be used for resistance monitoring.

Accessibility

Making these tools accessible to growers and researchers has been at the forefront of our planning. The equipment and skills required for the molecular biology and data analysis are prohibitive for wide use in the cotton industry. That's why we have negotiated with third party service providers who would be able to do the molecular biology and return the data promptly. We are also developing an easy-to-use data portal, where the data can be uploaded and 'with the click of a button' will be analysed and an allele frequency report returned to the user. This approach makes it possible to then develop a 'map' of resistance for individual farms or regions that is continually improved with up-to-date information and provides a clear visual depiction of resistance management.

This project is a proof-of-concept that we see being expanded and ultimately widely used by the cotton industry. If you have any comments or questions about the work we are doing, or how we are doing it, I would love to hear from you. I am especially interested in hearing from growers: Are you interested in using the tools we are developing? And what are the biggest challenges for pest management in an Australian cotton field? Contact Amanda Padovan E: amanda.padovan@csiro.au



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