FINAL REPORT

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Project Title: Validation and implementation of new molecular tools for Bt resistance monitoring
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Organisation: CSIRO

Confidential or for public release? For Public Release

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Summary for public release

Executive Summary	In this work we integrated, refined and validated molecular tools for Bt resistance monitoring in *Helicoverpa* spp. in Australian cotton fields. 1. We have simple easy to use workflows for Bt resistance allele detection that are very sensitive in a variable genome 2. A first pass population genetics study of *Helicoverpa armigera*, including the northern cotton growing regions and over time reveals no genetic differentiation at any spatial or temporal scale investigated here 3. We have identified putative Bt resistance alleles in laboratory-maintained, field-derived Bt resistant insect colonies using a quantitative genetics approach. Many of these live insect colonies are now being maintained at CSIRO Black Mountain. The molecular tools used here offer new insights into the genetic complexity of Bt resistance in *Helicoverpa* spp in Australian cotton systems, indicating the RMP strategies are highly effective at reduce the selection pressure on insects and preventing the evolution of resistance. However, in the context of Bt resistance, these molecular tools must be used in conjunction with traditional bioassays or phenotype-based resistance monitoring, as they are not effective at identifying novel Bt resistance causing mutations.
Objectives	Project outputs and outcomes will include: • Validate the molecular tool(s) for F1 and F2 resistance testing of H. armigera (and where feasible H. punctigera). • Strategic sampling during the validation study to provide the basis for a more detailed future study describing regional connectedness of

	H. armigera (and where feasible H. punctigera) as a foundation for understanding resistance evolution • Review the potential use of the molecular tool for R&D using historical collections and datasets with priority on informing RMP tactics (e.g. pupae busting, use of magnet, and refuges).
Background	In 2002 CSIRO initiated a program to monitor resistance in field populations of Helicoverpa spp. to the toxins (Cry1Ac and Cry2Ab) in second generation Bt cotton. We developed F2 screens which detected even recessive forms of resistance and detected resistance alleles for Cry2Ab prior to commercial release of cotton with this toxin. We characterised it, evaluated the risk of resistance developing to Cry1Ac through laboratory selection, and characterised and examined the implications of Bt resistance in *H. armigera*. We developed an abridged version of the F2 screen, called an F1 screen, which focussed on more efficiently estimating frequencies of that common resistance. In anticipation of the release of cotton carrying Vip3A we began screens for this protein and established a relatively high baseline frequency of resistance for both species. We characterised that resistance as high level, recessive, susceptible to Cry1Ac, and showing no major fitness costs. We proposed to intermittently perform F2 screens and routinely focus on the more efficient F1 screens to detect changes in frequencies of the resistances identified to date. We introduced a new component which allowed us to test for potential multi-resistances as well as novel dominant forms of resistance and formally incorporated an annual survey of Crop Consultant Australia members to assess the field performance of Bt cotton. From the outset of our involvement in Bt stewardship research, while developing an adaptive and robust monitoring program for the industry that is informed by characterisation of isolated resistances, we have overseen the co-evolution of sister programs conducted by Bayer. As part of its commitment to the control of Helicoverpa spp. in Australia, a strategic investment by CSIRO has sequenced the *H. armigera* and *H. punctigera* genomes. This enabled projects to identify the mechanisms of resistance and strategic investment in research on the potential to develop a molecular diagnostic tool for Bt resistance. This project brings together CSIRO, CRDC and Bayer to further develop and deploy these molecular tools using information and material gathered during the resistance monitoring and characterisation work since 2002. This project builds upon the molecular resistance monitoring tools developed in CSE1801 and integrates there use into the usual workflows at Bayer and CSIRO. The data generated is used to monitor for Bt resistance alleles across the Australian cotton growing regions and improve our understanding of the evolution and ecology of Bt resistance across the landscape.
Research activities	We validated and integrated the molecular Bt resistance monitoring tools developed in CSE1801 into existing workflows at CSIRO and Bayer. We also performed a first pass population genetics study to look at *Helicoverpa armigera* population structure across the entire cotton growing region. We used long read sequencing technologies to investigate Bt resistance mechanisms in our field derived, laboratory maintained Bt resistant insect colonies.

Outputs	This project revealed that. Bt resistance in Helicoverpa spp is caused by several genes and multiple alleles in each gene - this is much more complicated than we thought. This means two things: 1. that molecular resistance monitoring cannot replace conventional bioassay based monitoring approaches 2. the RMP is effective at preventing the evolution of Bt resistance in Helicoverpa spp. in Australia. The data generated within this project also suggests that Helicoverpa armigera is one large population across space and time, which means that individual growers pest management decisions could have implications throughout the entire industry.
Impacts	The outcomes of this project are already informing decision making at the TIMS committee and Bt tech panel. The support the notion that the RMP is highly effective at preventing the evolution of resistance, and that management decisions have the potential to impact the entire industry. The outcomes of this project will be published in peer-reviewed scientific journals and contribute to our growing understanding of the genetics of Bt resistance in Lepidopteran pests. Additionally, the outcomes will be shared through conference presentations and articles in the Spotlight magazine to reach a diverse audience.
Key publications	“Disruption of HaVipR1 confers Vip3Aa resistance in the moth crop pest Helivoverpa armigera”. “Sew what: cotton pest has jumper genes”. CONFIDENTIAL “Transposable element disruption of a second thyroglobulin-like gene confers Vip3Aa resistance in Helicoverpa armigera”.