Final Report

Management of insecticide resistance in serpentine leafminer (*Liriomyza huidrobrensis*)

Project leader:

Duong T. Nguyen

Delivery partner:

NSW Department of Primary Industries

Project code:

AS20002

Project:

Management of insecticide resistance in serpentine leafminer (*Liriomyza huidrobrensis*) (AS20002)

Disclaimer:

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Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

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Public summary

Serpentine leaf miner (SLM), *Liriomyza huidobrensis*, invaded New South Wales and Queensland late 2020. Overseas, SLM has a history of developing resistance to several chemical insecticides. Since its Australian discovery, SLM has caused significant control issues in Australian horticultural industries. The project aimed to develop and deliver cost-effective and accurate insecticide resistance surveillance tools and management strategies for SLM in Australia.

First, we developed efficient bioassay methods against both SLM larval and adult stages to test responses to insecticides. We found that dimethoate (Group 1) and imidacloprid (Group 4) were only able to control SLM at application rates of 33 and 200 times the field-recommended rates, respectively. Furthermore, we found that 64-fold the field-recommended rate of chlorantraniliprole (Group 28) controlled only 94% of one tested population. Similarly, spirotetramat (Group 23), tested at 64-fold the field-recommended rate, only achieved 84-94% mortality. As none of the above-tested insecticides will likely completely control Australian invasive SLM, the project made additional effort to find an insecticide that may work against SLM. Encouragingly, we found spinetoram (Group 5) fully controlled tested populations at a rate 160-640 times lower than the field-recommended rate.

The whole genome of Australian SLM was sequenced for the first time in partnership with a NSW DPI AUSGEM research grant. The SLM genome sequence facilitated designing a primer panel and the development of a multiplex amplicon sequencing platform for DNA-based target-site resistance causing detection. The DNA-based assay further supported the bioassay data by elucidating fixed resistance genes against several chemical insecticides, including Group 1 - Carbamates & Organophosphates; Group 2 - Cyclodienes and Fiproles; Group 3 – Pyrethroids, and Group 28 – Diamides. Notably, the current DNA assay did not detect Group 4 target-site resistance in Australian SLM, suggesting that other mechanism(s) are involved in imidacloprid's ineffectiveness.

The project has made a significant contribution to sustainable SLM management by providing a comprehensive understanding of the current status of insecticide resistance in Australian SLM populations. The successful sequencing of the SLM genome not only facilitated a DNA-based target-site resistance detection capability for Australian SLM but also provided a valuable DNA-based resource for researchers in the fields of insecticide resistance, population genetics and species diagnostics.

Successful resistance management of any arthropod species can only be achieved via rotation of compounds from different modes of action groups. For that reason, we strongly recommend expanded bioassay testing to include other permitted insecticide groups and biological products. This will significantly enhance a sustainable SLM management because the control strategy currently relies solely on the effectiveness of Spinetoram. Further, understanding the mechanism of spirotetramat resistance is a crucial step towards implementing a sustainable management strategy assuming reversion to susceptibility. Routine resistance surveillance using the DNA-based and bioassay testing developed by this study provides the industry with early signs of resistance, empowering them to take proactive management measures before consequences occur at the field level. With the above recommended tools and strategies, we are confident that Australian horticultural industries can effectively manage insecticide resistance in SLM thereby ensuring sustainability and productivity.

Keywords

Serpentine leaf miner, *Liriomyza huidobrensis*, insecticide resistance, bioassay, genome, target-site resistance, management strategy.

Introduction

Serpentine leaf miner (SLM), *Liriomyza huidobrensis*, is a tropical and warm temperate pest species that was restricted to central and South America until the 1980s (CABI 2021; Weintraub et al. 2017). Due to trade containing contaminated agricultural products, SLM has become a global pest, spreading to all continents, including Australia. It was first detected in Western Sydney, New South Wales (NSW), in October 2020 and one month later in southern Queensland (QLD) (EPPO 2021; Mulholland et al. 2022). The economic loss caused by SLM is not just cosmetic effecting quality standards for supermarkets and consumers. It's a serious threat that can lead to the total destruction of infected plants due to foliage damage. Consequently the resulting financial cost for growers can be substantial (Chiluwal et al. 2012), with crop yield reduced by up to 100% in potatoes (Alves et al. 2017; Kroschel et al. 2020; Shepard & Braun 1998). To prevent such economic loss, synthetic pesticides are used by growers to control the pest.

As SLM is a new invasive species to Australian horticulture, its chemical control and potential insecticide resistance are largely unknown, posing a significant threat to sustainability. Therefore, this pioneering project aimed to develop and deliver cost-effective and accurate insecticide resistance surveillance and management tools for SLM in Australia.

The project comprised five main activities: **(1)** Whole genome sequencing of SLM was done for the first time through PacBio long-read and Illumina short-read sequencing. Although funded by a separate NSW DPI AUSGEM research grant (led by Dr Grant Herron and titled 'Improved insecticide resistance detection in insects and mites via genomics') it was agreed in the AUSGEM planning process genomics outcomes could support related studies. This Horticulture Innovation funded project further facilitated the establishment of an inbreeding SLM line known as Gurner, which was originally collected from NSW; **(2)** Using the genome made available, target-site resistance genes were screened for in SLM samples collected from all geographic locations via our newly developed multiamplicon sequencing platform; **(3)** The first insecticide dose-responses and, as such, baseline responses were undertaken for SLM populations collected from NSW and QLD against some permitted insecticides used for their control, including dimethoate (Group 1 – Carbamates & Organophosphates), imidacloprid (Group 4 – Neonicotinoids), spirotetramat (Group 23 – Tetronic and Tetramic acid derivatives), chlorantraniliprole (Group 28- Diamides) and spinetoram (Group 5 – Spinosyns). Prior to this study, the efficacy of chlorantraniliprole and spirotetramat against leaf miner was poorly studied, with only a few reports with chlorantraniliprole against American SLM; **(4)** Glasshouse trials were undertaken for four chemicals (spirotetramat, chlorantranliniprole, dimethoate and imidacloprid) against a SLM population at Gatton Research Station, Queensland Department of Agriculture and Fisheries (QDAF). The QDAF trials support the NSW DPI bioassay and DNA reports of resistance in SLM that resulted in complete control failures; **(5)** Extension activities were undertaken in conjunction with project MT20005, AusVeg and NSW Local Land Services. Research outputs and outcomes have been disseminated to Australian researchers, agronomic consultants and growers in the Northern Territory, Western Australia, Queensland, and News South Wales.

The outputs and outcomes of this project have become an integral linkage to Hort Innovation projects, MT20005 and MT16004, for the development of a SLM management strategy. The Hort. Frontiers Advanced Production Systems Fund aims to "Increase productivity and profitability of Australian horticulture through cropping system intensification and innovation programs targeting the whole of horticulture". In support, we have provided sustainable and scientifically

grounded management decisions for SLM. This approach has been instrumental in minimising production loss for Australian horticulture. The successful finishing of this project additionally has contributed to:

- Vegetables strategic investment plan 2022-2026: Outcome 1 Strategy 2 and the KPI - Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers.
- Potato strategic investment plan 2022-2026: Outcome 2 Strategy 1 and the KPI-Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers.
- Onions strategic investment plan 2022-2026: Outcome 2 Strategy 1 and the KPI-Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.
- Melon strategic investment plan 2022-2026: Outcome 2 Strategy 2 and the KPI-Development of pest and disease management strategies that mitigate crop loss in collaboration with growers.
- Nursery strategic investment plan 2022-2026: Outcome 2, strategy 2&8 - Improve industry biosecurity preparedness and resilience, including prevention, protection and recovery from exotic and endemic plant pest incursions and responses and Develop and optimise fit-for-purpose integrated pest and disease management (IPDM) strategies for growers.

Methodology

Component 1: Establishment of SLM strains at EMAI

Leaves with leaf-mining damage were collected directly from fields in NSW and QLD. Briefly, each collection included 20-30 infested leaves randomly gathered throughout the crop on the same day. Leaves with leaf-mining damage were packaged and transported via overnight courier to the Elizabeth Macarthur Agricultural Institute at Menangle, NSW for processing. A minimum of 50 pupae were used from each forwarded collection to set up a subsequent strain.

All SLM strains were maintained on potted French bean (*Phaseolus vulgaris* L.) under insecticide-free conditions in an insectary at 25±2°C and isolated in insect-proof cages to ensure strain integrity.

Component 2: Bioassays

Chemical tested

Imidacloprid 200 g/L (Confidor® 200 SC insecticide) and spirotetramat 240 g/L (Movento® 240SC insecticide) were supplied by Bayer Cropscience Australia. Dimethoate 400 g/L (Dimethoate 400) was supplied by Adama Australia Pty Ltd. Chlorantraniliprole 200 g/L (Coragen® insecticide) was supplied by FMC Australasia Pty Ltd. Spinetoram 120 g/L (Success ™ Neo insecticide) was supplied by Corteva Agriscience Australia Pty Ltd. These insecticides are permitted under minor use permits for SLM control in Australia. Chlorantraniliprole and spinetoram were tested in addition to agreed milestones to provide industry data for potentially useful Group 28 Diamide and Group 5 Spinosyn insecticides.

Two NSW SLM strains (Kemp C and Riley-2) and one Queensland SLM strain (Wyreema-2021) were selected for bioassay. Other established SLM strains were tested for resistance genes through genetics (Appendix A - Table A1).

Spinetoram was tested against NSW strain, Kemp C, and Queensland strain, Wyreema-2021.

Adult bioassays

As imidacloprid, dimethoate, spinetoram and chlorantraniliprole are systemic insecticides with contact and stomach action they were tested against adults. Briefly, three young female flies (~5 days old) were lightly anaesthetised with CO_2 and tipped onto the French bean leaf disc. The Petri dish containing anaesthetised flies was sprayed with serial concentrations of aqueous formulated insecticide (2 mL) imidacloprid, dimethoate, spinetoram or chlorantraniliprole with the aid of a Potter spray tower producing a 1.5-1.7 mg/cm^2 aqueous deposit. Each bioassay replicate comprised 4–5 serial concentrations, with 5-7 sprayed Petri dishes sub-batches at each test concentration (i.e., 15 to 21 flies). Each full bioassay test was replicated three to six times on different days and included a water only sprayed control that did not exceed 15% mortality. Chlorantraniliprole was additionally diluted with Agral spray adjuvant at the rate of 0.025 mL/100 mL as a wetter that also produced control mortality of less than 15%. After spraying, the Petri dishes with flies were maintained at 25 ±2 ^oC in 16:8 light: dark condition for 48 h, after which mortality was assessed. Data were control corrected (Abbott 1925) and subjected to probit analysis, and LC_{50} and $LC_{99.9}$ values and their associated 95% fiducial limits (FLs) calculated (Finney 1971) via a purpose written software program (Barchia 2001).

Larval bioassays

Spirotetramat affects lipid biosynthetics, penetrates the leaf tissue, and is transported within both the xylem and phloem. Therefore, this two-way systemic insecticide is more effective in controlling insect juvenile development stages (Nauen et al. 2008). Thus, spirotetramat bioassays were performed against SLM larval stage only.

Firstly, French bean plants were placed in an insect cage infested with 150-200 SLM adults (4-5 days old) for 3 hours. Next, leaves were cut from the plants and the number of eggs per leaf was counted using a stereo microscope with inverted light.

Next individual leaves were sprayed with a serial concentration of aqueous spirotetramat (2 mL) diluted with Hasten® spray adjuvant at 0.05 mL/100 mL. Each concentration sprayed included an average of 30 eggs deposited in about 5-6 leaves. Each full serial concentration dose response test was replicated 4 times on different days and included a Hasten® only (0.05 mL/100 mL) control that did not exceed 15% mortality. After spraying, each individual leaf was kept in a Petri dish (diam. x 140 mm x 20 mm) with filter paper lining. The number of hatched larvae from each leaf was confirmed after 52 hours. The test was maintained at 25 ±2 ^{o}C in 16:8 light:dark for two weeks. Mortality was assessed as the number of larvae that failed to pupate. Data analyses were as for the adult bioassay above.

Component 3. Genomic sequencing of SLM (partly funded by AUSGEM)

Establishment of inbreeding SLM strain

To facilitate a high-quality genome sequence for Australian SLM, we established an inbred SLM strain through single-sibling mating that went for 4 generations.

First generation F0 female and male parents were originated from the Gurner SLM strain collected from Sydney basin (Appendix A - Table A1). Individual F0 pairs were reared on a French bean plant in a plastic box(s) (275 x 275 x 263 mm) with lids including an insect proof mesh (mesh aperture 160 µm) for ventilation. F1 pupae were collected into glass vials to await adult emergence. Subsequent F1 adult flies were sexed and separated as soon as they emerge to ensure their virginity. Virgin F1 sisters were paired individually with their F1 brother to obtain an F2 generation. This process was repeated for four generations to create the inbred strain.

Whole genome sequencing to identify insecticide resistance target genes

High-molecular-weight DNA and purified RNA were produced from the established inbred SLM adult flies. This was sent to DNA Link Inc. for PacBio long-read sequencing and Illumina short-read sequencing. A de novo transcriptome assembly was produced by Trinity 2.14.0. A list of transcripts that contain the target genes was identified by tblastn (NCBI blast plus). The whole transcriptome RNA-seq sequence was aligned to a candidate reference sequence (*Drosophila melanogaster*) with SNP call carried out by Freebayes. To validate gene sequence, we searched the ORF for matches against the InterPro protein signature databases InterPro 98.0 (https://www.ebi.ac.uk/interpro, accessed on 7 March 2023) using InterProScan tool (Paysan-Lafosse et al. 2022). Additionally, we performed multiple sequence alignments of protein sequences using Cobalt (Papadopoulos & Agarwala 2007). The full CD gene sequence of insecticide target resistance genes in Australian SLM were submitted to NCBI GenBank.

Component 4. Insecticide resistance target sites investigated via massive parallel sequencing

DNA extraction

234 DNA samples were extracted from individual flies collected from NSW and Queensland (Appendix A - Table A1) by Chelex 5% (Sigma) according to the protocol developed by Entomology Insecticide Resistance & Genomics team.

Primer design

A primer panel amplifying multiple target-site mutations in SLM were successfully designed (Appendix A - Table A2). Multiplex PCR assays for resistant gene screening were completed with the support of the handling robot epMotion at the EMAI Advanced Gene Tech Center. A final library amplified 6 target-site mutation points causing resistance and a mitochondrial gene was processed from 234 flies. Pooled library was sequenced by the MiSeq Illumina facility located at EMAI. The analysis of multiamplicon sequencing data involves several steps, including demultiplexing initial sequencing data, quality assessment and trimming, merging paired-end reads, aligning sequence to reference and variant calling.

Component 5. Field trials and extension support

Field trials

Glasshouse trials were undertaken for spirotetramat, chlorantraniliprole, dimethoate and imidacloprid against SLM at the Gatton Research Station, Queensland Department of Agriculture and Fisheries (QDAF).

In brief, two French bean plants were included per trial pot. Field recommended rates of dimethoate, spirotetramat, chlorantraniliprole and imidacloprid were tested. Each insecticide trial was replicated twice. One replicate included three cages, each containing one pot (two plants) sprayed with a tested insecticide and another a water/adjuvant control. Treatments were sprayed using a cone nozzle making sure all the leaves were covered to run off. Treated plants were left to dry for a couple of hours in domed cages in the laboratory before being moved into the cage accommodating control and treated potted plants. Next, 30 5-day-old SLM adults (10 males and 20 females) were released into each cage and left for 48 hours. After that, adult flies were removed from the cages, and deaths and survivors were recorded. Five days after removing the flies, two fully expanded leaves from each plant were removed to count the number of mines (mining tracks caused by larvae) produced by newly hatched larvae. Leaves were then maintained in petri dishes with moist filter paper until pupation. Final adults produced were recorded after all flies had emerged, usually by two weeks.

To determine statistical significance, an independent t-test was used via GraphPad Prism 8.4.3 to determine the significant difference in the number of mines, pupae and adults between control and insecticide treated plants.

Extension activities

The activities were facilitated via MT20005 and its established extension pathways, see more details MT20005 final report, component 4 and 8. This included AusVeg, the Hort Innovation Extension Team and existing vegetable communications networks. Workshops face-to-face, farm visits, field days and other engagement activities were organised by MT20005. The project leader attended one workshop in the Northern Territory and a Field Day at Gatton Research Station to give Australian Researchers, agronomists, consultants and growers SLM management strategy based on the project results. The flyers (Appendix C) and presentations containing insecticide resistance status were provided to other workshops.

Results and discussion

Component 1: Establishment of SLM strains at EMAI

Nine strains collected from NSW and two from QLD were established (Appendix A - Table A1). Species identification was confirmed via PCR amplification of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene by the primer pair, LCO1490 and HCO2198 (Folmer et al. 1994). *COI* amplified sequences (658 bp) of collected SLM strains were identical to the SLM *COI* sequence published in the GenBank (accession number KC136091.1).

Component 2: Bioassays

Adult bioassay

Dimethoate and imidacloprid

Baseline data generated suggested the two tested NSW and one Queensland SLM strains are homogeneously resistant to both dimethoate and imidacloprid. LC_{50} estimates for NSW and Queensland ranged from 1.4 (95%FL 0.8-2.0) to 2.5 (95%FL 2.1-3.0) g ai L^{-1} for dimethoate and 1.8 (95%FL 1.0-2.8) to 2.0 (95%FL 1.7-2.4) g ai L^{-1} for imidacloprid. The slope value obtained for Queensland strain Wyreema-2021 against dimethoate was lower than 2, indicating the response is more heterogeneous than for NSW strains Kemp C and Riley-2 (Yu 2008). Interestingly, in a glasshouse experiment, a potted bean plant had its leaves seriously burnt by a 4 g ai L^{-1} dimethoate sprayed, yet flies released on this plant still survived and successfully produced offspring (Appendix A - Figure A2). The log-dosage probit (LDP) curves obtained for all tested strains from both states against imidacloprid are steeper, with slope values ranging from 3.0 to 3.7, indicating a homogeneous response of tested strains against this chemical (Appendix A-Figure A1. A and B).

A comparison of laboratory data generated to the field rates for dimethoate and imidacloprid suggests both will be ineffective when applied at their approved permit rates. Ten g ai L^{-1} dimethoate (33-fold higher than the field rate) was required to achieve 100% control of strains Wyeerma-2021 and Kemp C, but that dose only controlled 97% of strain Riley-2 (Appendix A-Figure A1. A). Similarly, 10 g ai L^{-1} imidacloprid (200 times the field-recommended rate) controlled only 97% of strains Wyreema-2021 and Kemp C and 98% of strain Riley-2 (Appendix A-Figure A1. B).

Spirotetramat and chlorantraniliprole

Suggesting resistance, both chlorantraniliprole and spirotetramat did not fully control any of the tested SLM strains at substantially greater rates than the field-recommended rate. Dose responses of tested strains against chlorantraniliprole and spirotetramat all produced flat LDP curves. LC_{50} estimates ranged from 0.02 (95%FL 0.006-0.04) to 0.1 (95%FL 0.04-0.2) g ai L^{-1} for chlorantraniliprole and 0.2 (95%FL 0.1-0.5) to 0.5 (95%FL 0.2-1.6) g ai L^{-1} for spirotetramat. Slope values for all tested strains against these two chemicals were low, ranging from 0.7-1.23, indicating heterogeneous responses (Appendix A – Figure A1. C and D).

NSW strains, Kemp C and Riley-2, appeared to be more resistant to chlorantraniliprole than Queensland strain Wyreema-2021. A dose of 6.4 g ai L^{-1} (64 times the field rate) suppressed 94% of strain Kemp C and 100% of Riley-2. In contrast,

chlorantraniliprole was able to control 100% of Queensland strain Wyreema-2021 at a lower concentration of 1.6 g ai L^{-1} (4 times lower) (Appendix A – Figure A1. C). The three tested strains showed a similar pattern towards spirotetramat in which Queensland strain Wyreema-2021 was slightly less resistant than NSW strains Kemp C and Riley-2. Specifically, 6.4 g ai L^{-1} spirotetramat (64 times the field rate) controlled 94% of Queensland strain Wyreema-2021. On the other hand, this same dose could only suppress 84% of NSW Riley-2 and 92% of Kemp C (Appendix A – Figure A1.D).

Spinetoram

Baseline dose-response data against spinetoram tested on Queensland strain Wyreema-2021 and NSW strain Kemp C are encouraging. Spinetoram is the only tested chemical here to fully control tested SLM strains at doses 160-640 times lower than the field recommended rate. LC_{50} estimates ranged from 0.0007 (95%FL 0.0006-0.0009) to 0.003 (95%FL 0.003-0.004) g ai L^{-1}. The Log Dose Probability curves obtained for all tested strains against spinetoram are steep with slope values ranging from 3.8 to 4.3. This indicates a homogeneous response that we interpret as susceptible against this chemical (Appendix A – Figure 1. E).

Component 3. Genomic sequencing of SLM (partly funded by AUSGEM)

High-molecular-weight DNA and high-quality and purified RNA samples were extracted from a pool of inbred SLM adults. In total, we obtained clean data of 20 GB (90x coverage) from Illumina short-read and 22.5 GB (100x coverage) from PacBio HiFi long-read systems. We produced a high-quality SLM genome assembly by combining the short and long-read sequencing technologies. The final SLM genome size is 221MB. The genome assembly contained 532 contigs, with a contig N50 value of 902kb. Our SLM genome assembly contains 98.17 % Insecta BUSCO genes, which suggests a quality and complete genome.

In total, we obtained 5.6 Gb of clean RNA-sequence data, consisting of 55,464,148 Illumina short-reads. The transcriptome assembly generated 22,737 gene clusters, with a total of 34,536 transcripts. We identified candidate gene clusters for insecticide resistance target genes of acetylcholinesterase (*AChE*), voltage-gated sodium channel (*VGSC*), gamma-aminobutyric acid receptor (*RDL*), nicotinic acetylcholine receptor alpha 6 subunit (*nAChRα6*), nicotinic acetylcholine receptor subunit (*nAChRβ1*), chitin synthase 1 (*CSH1*), GluClalpha (*GluCl*), and ryanodine receptor (*RyR*). We identified the full coding sequences and annotated the predicted proteins with blastp and InterProscan. The fully annotated CDS for each gene was submitted to NCBI (Appendix A – Table A3).

Importantly, we are the first to report that six target mutations are present in Australian invasive SLM. Transcriptome analysis indicated Australian SLM carried three known target mutations (I129V, G227A and F331W) on the *AChE* gene causing both Organophosphate and Carbamate insecticide resistance; two known target site resistance mutations (M918T and L1014F) on *VGSC* gene causing resistance to Pyrethroids; one known target site mutation, A301S, Gamma-aminobutyric acid receptor encoded by the gene *RDL* conferring resistance to Cyclodiene and Fiproles; one known target site mutation, I4790M, on the *RyR* gene conferring resistance to Diamides. The two following known target site mutations were not detected: G275E on the *nAChRα6* associated with Spinosyn resistance and R81T on *nAChRβ1* associated with Neonicotinoid resistance (Appendix A – Table A4).

Component 4. Insecticide resistance target sites investigated via massive parallel sequencing

Our multi-amplicon sequencing platform successfully amplified the target genes, including *COI* (for species identification), *AChE, VGSC, nAChRβ1, CSH1, GluCl,* and *RyR* from the 234 DNA samples in order to survey resistance across Australian populations. On average, we obtained 5370 to 7993 high-quality sequences per sample across 7 amplicons, with a median from 4557 to 7461. We used the threshold of 50 reads for a valid PCR amplification.

Sequencing analyses resulted in the following resistant alleles being detected: I129V and G227A conferring resistance to Group 1A Carbamates (e.g. Methomyl, Carbaryl) and Group 1B Organophosphates (e.g. Chlorpyrifos, Dimethoate, Trichlorfon); Super kdr resistant alleles, M918T and L1014F, conferring resistance to Group 3A Pyrethroids (e.g. Bifenthrin, Permethrin, Alpha-cypermethrin, Zeta-cypermethrin, Cyhalothrin); I4790M mutation conferring resistance to Group 28 Diamides (e.g. Chlorantraniliprole, Cyclaniliprole, Cyantraniliprole). We detected no mutations associated with the Group 5 Spinosyns (e.g. Spinetoram, Spinosad), Group 6 Avermectins (e.g. Abamectin, Emamectin benzoate) or the Group 10B (e.g. Etoxazole) insecticides (Appendix A – Table A4).

Worryingly, all tested SLM populations were homozygous resistant to the detected point mutations indicating those mutations were fixed in Australian SLM.

Component 5. Field trials and extension support

Field trials

To support the bioassay and DNA technology outputs by the NSW DPI Insecticide Resistance & Genomics laboratory, glasshouse trials spray trials with field recommended rates of dimethoate, imidacloprid, spirotetramat and chlorantraniliprole were undertaken at the Gatton Research Station by QDAF.

Dimethoate

Glasshouse trials found no significant difference in response of SLM sprayed with dimethoate at the recommended field rate and with water only control. Confusingly, the number of mines, pupae and adults produced by SLM feeding on dimethoate treated plants for 48 hours appeared higher but was not significantly different from the control (Appendix A - Figure A3). The result supports the bioassay and DNA based conclusion that Australian invasive SLM are dimethoate resistant.

Imidacloprid

Glasshouse trials by QDAF confusingly found the number of mines, pupae, and adults that emerged from imidacloprid-treated plants actually appeared higher than those sprayed with a water-only control, but the difference was not statistically significant. (Appendix A - Figure A4). Nonetheless again the result supports the bioassay and DNA based conclusion that Australian invasive SLM are imidacloprid resistant.

Spirotetramat

Similarly, glasshouse trials by QDAF using the field recommended rate of spirotetramat did not significantly reduce the number of SLM mines, pupae and adults. The result supports the bioassay and DNA based conclusion that Australian

invasive SLM are spirotetramat resistant (Appendix A - Figure A5).

Chlorantraniliprole

Again, glasshouse trials by QDAF showed a potential resistant response of tested SLM against the chlorantraniliprole field recommended rate. Confusingly, the number of mines, pupae and adults produced in treated plants appeared higher than in the control although results are not significant (Appendix A - Figure A6).

Extension

- The principal investigator, Dr Nguyen attended a series of workshops organised by QDAF (MT20005) and AusVeg: **1)** Presented project findings on SLM insecticide resistance to farmers/growers, consultants and researchers at Berrimah Farm Science Precinct, Darwin on 6 July 2023. **2)** Contributed a presentation on SLM insecticide resistance to other AusVeg workshops, including a workshop in Kununurra at Frank Weiss, Western Australia, on 19 October 2023 **3)** and a similar workshop in Bundaberg, Queensland, on 8 November 2023.

- Dr Nguyen attended the Gatton AgTech Showcase on 1- 2nd November 2023. Here, Dr Nguyen showcased the latest insecticide resistance research results on SLM. Results were further relayed to Hort Innovation Australia, researchers, agribusinesses, chemical companies, consultants, horticulture growers and students across Queensland and around Australia. Presentations and one-page flyers (Appendix C) delivered by Dr Nguyen gained significant interest, as demonstrated by all flyers being sold out on the second day.
- Dr Nguyen attended the Australian Entomological Society 54th Annual General Meeting and Scientific Conference from 12 - 15 November 2023, Albany, Western Australia. Here, Dr Nguyen talked in the session "Pest management and control" with a presentation titled "Insecticide resistance in Australian serpentine leaf miner, *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae)".
- Dr Nguyen attended the Greenhouse Vegetable Farm Walk organised by Local Land Services NSW and Protected Cropping Australia at Kemp Creek, NSW on 28th November 2023. Here she highlighted project findings on SLM insecticide resistance to growers, consultants and researchers.

Outputs

Table 1. Output summary

Output	Description	Detail
SLM strains for resistance testing at EMAI	Nine strains from NSW and 2 strains from QLD were established for resistance testing.	Two NSW strains Kemp C and Riley-2, and one QLD strain Wyreema-2021, are in culture at EMAI for resistance testing. Other strains were ethanol preserved for genetic resistance surveillance. See Appendix A - Table A1. See Milestone report 103.
A SLM Inbred strain	One inbred SLM strain established for genome sequencing	An inbred strain called Gurner, which was originally collected from NSW, was established by single-sibling mating for 4 generations. The inbred strain is in culture at EMAI. Adults of this strain were collected for high-molecular-weight DNA and high-quality RNA extraction for genome sequencing. See Milestone report 104.
Resistance baseline data for SLM against dimethoate, imidacloprid, and spirotetramat	Three resistance baseline data sets generated.	Resistance monitoring data generated for two NSW SLM strains and one QLD strain against dimethoate, imidacloprid, spirotetramat. NB in addition to the contracted milestone we also did chlorantraniliprole and spinetoram via adult bioassay. This would ultimately become the foundation for our resultant SLM management strategy. See Appendix A - Figure A1 A,B,C,D &E. See Appendix B - Manuscript submitted to *Austral Entomology*, Manuscript ID: AEN-6168. See Milestone report 106.
Glasshouse trial data efficacy data for the field recommended rates of dimethoate, imidacloprid, spirotetramat	Three data sets generated for the efficacy of the field recommended rate of dimethoate, imidacloprid, spirotetramat.	The three data sets generated to validate NSW baseline response data. See Appendix A - Figure A3,4&5. Milestone report 107. And addition to the contracted milestone: chlorantraniliprole against SLM, supporting NSW DPI baseline data See Appendix A – Figure A6.
A high quality Australian SLM draft genome sequence	A high-quality genome SLM assembly generated.	This work was funded by the NSW DPI AUSGEM research grant led by Dr Grant Herron. We produced a high-quality genome SLM assembly by combining the short-read and long-read sequencing technologies. The final SLM genome size is 221MB. The genome assembly has 532 contigs, with a contig N50 value of 902kb. Our SLM genome assembly contains complete 98.17 % Insecta BUSCO genes, which indicates sufficient quality and completeness of the genome. This assembly provides a valuable reference for all Australian researchers who are studying SLM, such as population genetics and molecular species diagnostics.

		See Milestone report 105. A manuscript is in preparation to be submitted to the scientific journal *Insects.* Sequencing data submitted to the GenBank and being published in a peer-reviewed journal.
Full gene sequences of known insecticide resistance genes	A list of insecticide target genes identified.	A list of insecticide target genes identified by full-length transcriptome sequencing analysis, becoming the foundation for subsequent DNA-based resistance detection. See Appendix A-Table A3. A manuscript is in preparation to be submitted to the scientific journal *Insects.* See Milestone report 106. Gene sequences were submitted to the GenBank and a manuscript being submitted to the scientific journal *Insects*.
A primer panel for target site resistance diagnostic assay(s)	A primer panel for target site resistance diagnostic assay(s) designed and generated.	Based on the generated genome sequence, the primer panel was designed and generated to identify potential target-site insecticide resistance alleles in Australian SLM. This is currently being prepared for publication. See Appendix A-Table A2. A manuscript is in preparation, being submitted to the scientific journal *Insects.* See Milestone report 106.
A multi-amplicon sequencing platform for target-site resistance gene identification	The sequencing platform designed and troubleshot.	A high-throughput and cost-effective sequencing platform was designed and worked based on the principle platform designed for fall armyworm which was previously published by us (Chen et al. 2023). As a result, a list of insecticide resistance genes was detected in Australian invasive SLM. See Appendix A-Table A4. See Milestone report 106. A molecular diagnostic tool to detect insecticide resistance in SLM via DNA technology developed: A manuscript is in preparation, being submitted to the scientific journal *Insects.* This tool and its results will be published in a peer-reviewed journal.
Industry news on AUSVEG	The news was published on 22 Feb 2022	Industry news published titled “Insecticide resistance detected in serpentine leafminer – Groups 1 and 3”. See https://ausveg.com.au/articles/insecticide-resistance-detected-in-serpentine-leafminer-groups-1-and-3/
Insecticide resistance status guideline	One-page flyer created to inform Hort Innovation Australia, researchers, agribusinesses, chemical companies, consultants, horticulture growers	The insecticide resistance status of Australian serpentine leaf miners was summarized in a one-page flyer and disseminated in the Field Days and workshops as a part of extension activities organised by MT20005 & AUSVEG, NSW Local Land Service (NSW LLS). See Appendix C.

	and students across Queensland and around Australia about serpentine leaf miner insecticide resistance status	
Project Monitoring & Evaluation Plan	Covers Program Logic, Key Evaluation Questions, and Performance expectations, data collection and analysis	See Milestone 102.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
A greater understanding of current status of insecticide resistance in Australian SLM populations	1, Vegetables Outcome 1 Strategy 2 and the KPI - Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers. 2. Potatoes Outcome 2 Strategy 1 and the KPI Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers. 3. Onions Outcome 2 Strategy 1 and the KPI Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases 4. Melons Outcome 2 Strategy 2 and the KPI Development of pest and disease management strategies that mitigate crop loss in collaboration with growers 5. Nursery Outcome 2 Strategy 8 and the KPI Develop and optimise fit-for-purpose integrated pest and disease management (IPDM) strategies for growers	Growers and agronomists are now informed of the efficacy of permitted chemicals and what chemicals should be included in the management strategy.	Milestone 106 & 107. Journal articles on current status of insecticide resistance in Australian SLM populations (Appendix B and another in preparation being submitted to the scientific journal *Insects*). One-page flyer released in the Field days and workshops organised by MT20005, AUSVEG and NSW LLS (Appendix C).
Greater understanding of SLM genome providing a valuable DNA base resource for all Australian researchers in the field of insecticide resistance, population genetics and species diagnostics		Australian researchers in insecticide resistance, population genetics, and species diagnostics can now use the generated Australian SLM genome for their research programs.	Genome sequence submitted to GenBank. Journal articles on SLM genome and SLM resistance profile (A manuscript in preparation being submitted to the scientific journal *Insects*).
Diagnostic tools developed to detect insecticide resistance in SLM via bioassay and DNA technology		The developed bioassay and molecular diagnostic tools can be used for annual surveillance of insecticide resistance in Australian SLM populations. This provides insecticide resistance information to related industries before consequences are seen at the field level.	Journal articles on baseline response data, SLM genome and SLM resistance profile. (Appendix B and another in preparation being submitted to the scientific journal *Insects*).
The study helps reduce the impact of SLM in horticultural crops achieved via a sustainable insecticide resistance strategy, bioassay and DNA based insecticide resistance diagnostic tools.		Resistance baseline data, genome sequence, and molecular surveillance tools generated by this study lay the groundwork for the SLM management strategy.	Journal articles on baseline response data, SLM genome and SLM resistance profile. Industry update workshop facilitated by Hort Innovation, AUSVEG and their extension network. (Appendix B and another in preparation being submitted to the scientific journal *Insects*).

Monitoring and evaluation

Table 8. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Effectiveness 1. To what extent has the project achieved its expected outcomes?		
End of project outcomes The study helps reduce the impact of SLM in horticultural crops achieved via a sustainable insecticide resistance strategy, bioassay and DNA based insecticide resistance diagnostic tools.	Project successfully provided a comprehensive understanding of the current status of insecticide resistance in Australian invasive SLM, plus also equipped them with efficient bioassay and DNA-based resistance testing tools. These tools developed in this study, will allow for the first time chemical resistance monitoring of SLM.	
Did the project identify the status of insecticide resistance in Australian SLM populations?	• Insecticide resistance status of Australian SLM was successfully generated via bioassay and DNA-based technology. Baseline data established against three SLM strains to dimethoate (Group 1 -Carbamates & Organophosphates), imidacloprid (Group 4-Neonicotinoids), spirotetramat (Group 23-Tetronic/tetramic acid derivatives), and addition to the contracted milestone: chlorantraniliprole (Group 28-Diamides) and spinetoram (Group 5-Spinosyns). A manuscript submitted to Austral Entomology, currently under review, manuscript ID AEN-6168 (Appendix B). • Additionally, DNA based resistance testing further confirmed resistance status of Australian SLM. It identified resistance alleles against Group 1 -Carbamates & Organophosphates; Group 2 - Cyclodienes and Fiproles; Group 3 – Pyrethroids, Group 28 – Diamides. A manuscript in preparation, being submitted to the scientific journal	• Bioassays to assess the efficacy of environmentally friendly products, including Azamax, SeroX, and a *Bacillus thuringiensis* product (Group 11A), and other chemical insecticides that have not been tested, including Abamectin, Emamectin benzoate, Cyromazine, and an unregistered product, Simodis (Group 30 – 100g/L Isocycloseram). • Study the mechanism of Group 23, spirotetramat resistance and fitness cost of resistant SLM. The results of this study will be instrumental in implementing a sustainable management strategy to revert spirotetramat resistance to susceptibility. • Undertake routine DNA-based resistance testing using the multiplex amplicon sequencing platform developed by this study to provide the industry with early information about resistance before the consequences are seen at the field level.

	Insects.	
Did the project develop a sustainable IPM based insecticide resistance management strategy for SLM?	Baseline data and DNA-based resistance testing suggested that Spinetoram and potential Group 6 insecticide Avermectins can be included in the management strategy, as known target site resistance genes associated with these chemical groups were not detected.	Bioassays to assess the efficacy of environmentally friendly products, including Azamax, SeroX, and a *Bacillus thuringiensis* product (Group 11A), and other chemical insecticides that have not been tested, including Abamectin, Emamectin benzoate, Cyromazine, and an unregistered product, Simodis (Group 30 – 100g/L Isocycloseram).
Did the project develop a molecular diagnostic tool to detect insecticide resistance in SLM?	A primer panel and a multi-amplicon sequencing platform were successfully developed. A manuscript in preparation, being submitted to the scientific journal *Insects.*	Molecular-based resistance testing is more cost-effective than bioassay; therefore, there is significant potential to transition from bioassay to routine resistance detection through DNA-based monitoring.
Did the partner project AUSGEM produce SLM genome assembly?	Australian SLM genome assembly has been successfully done, serving as the basis for the identified target sequences and their associated resistance mutations. The genome assembly sequences have been submitted to GenBank for researchers for utilization.	The genome of the American SLM has not been sequenced, which has hindered the development of DNA-based resistance testing for this species. The NSW DPI team can conduct a study by first sequencing the whole genome and then developing a DNA-based sequencing platform for resistance monitoring.
Did the project undertake a field trial and a field day for extension?	The glasshouse trial data generated by QDAF met the expected outcome supporting NSW DPI baseline response data validation.	The established methodology of glasshouse trials will be used alongside bioassays for validation purposes.
Relevance **2.** How relevant was the project to the needs of intended beneficiaries?		
Did the project develop an insecticide resistance management plan for the growers use to control SLM?	Baseline data and DNA-based resistance testing suggested that Spinetoram and potential Group 6 insecticide Avermectins can be included in the management strategy, as known target site resistance genes associated with these chemical groups were not detected.	Bioassays to assess the efficacy of environmentally friendly products, including Azamax, SeroX, and a *Bacillus thuringiensis* product (Group 11A), and other chemical insecticides that have not been tested, including Abamectin, Emamectin benzoate, Cyromazine, and an unregistered product, Simodis (Group 30 – 100g/L Isocycloseram).

Did the project receive SLM genome assembly data from the AUSGEM partnership for researchers to use for SLM research purposes?	Australian SLM genome assembly has been successfully completed, serving as the basis for the identified target sequences and their associated mutations. The genome assembly sequences have been submitted to GenBank for researchers' utilization.	
Process appropriateness 3. How well have intended beneficiaries been engaged in the project?		
Have regular project updates been provided through linkage with the industry communication project? E.g. AUSVEG, NSW DPI media team	Project outputs and outcomes have already been well disseminated via published industry news (AUSVEG), Field days and workshops organised by MT20005, the Australian Entomological Society conference, and NSW DPI website.	
Did the growers and consultants attend the field day and take the benefit from this activity combined with field trial to understand insecticide resistance management strategy?	Growers and consultants expressed strong interest in the study's findings presented during the Field Days and workshops. For more details, refer to the final report of MT20005.	
4. To what extent were engagement processes appropriate to the target audience/s of the project?		
Were project outcomes provided in a readily accessible form to stakeholders?	• A manuscript was under peer journal review. • Another manuscript was in preparation. • Presentation in the industry workshops facilitated by MT20005 and AUSVEG. • A one-page flyer was disseminated in all related industry workshops facilitated by MT20005 and AUSVEG.	Continued Engagement
How accessible was the field day as extension event to industry levy payers?	The Gatton AgTech Showcase on 1-2nd November 2023, organised by QDAF was a free entry event. Here, the principal investigator showcased the latest insecticide resistance research results on SLM. Results were further relayed to Hort Innovation Australia, researchers, agribusinesses, chemical companies, consultants, horticulture growers and students across Queensland and around Australia.	Continued Engagement

Was the information (media release, industry articles…) presented in a way that was useful to growers?	• The project update is easily accessible on the Hort Innovation website. • The one-page flyer effectively summarizes the insecticide resistance status of Australian SLM.	Continued Engagement
Efficiency 5. What efforts did the project make to improve efficiency?		
What efforts did the project make to improve efficiency?	• Whole genome sequencing of Australian SLM was achieved in partnership with an NSW DPI AUSGEM research grant titled 'Improved insecticide resistance detection in insects and mites via genomics'. The inclusion of AUSGEM provided significant real project cash value and formed the foundation of a comprehensive DNA based resistance detection capability for Australian SLM. • The principal investigator efficiently worked with MT20005 to disseminate the project findings in a timely manner via their extension activities and network.	
What has the project achieved to assist growers manage insecticide resistance in SLM?	This project has provided horticultural growers in Australia with valuable insights into the current status of insecticide resistance in the invasive SLM populations. It has also informed them about potential chemical insecticides that can be incorporated into the SLM management strategy.	Continued resistance testing through bioassay and DNA-based methods will be vital to offer growers more options of biological products or potential chemical insecticides for SLM management.

Recommendations

This study provides Australian horticultural industries with a comprehensive understanding of the current status of insecticide resistance in Australian invasive SLM, plus also equipped them with efficient bioassay and DNA-based resistance testing tools. These tools developed in this study, will allow for the first time chemical resistance monitoring of SLM. This empowers the industry to effectively manage insecticide resistance in SLM, thereby ensuring the sustainability and productivity of affected horticultural crops. Future opportunities include:

- Successful resistance management requires the rotation of compounds from different modes of action groups, the more the better. Therefore, it is crucial to expand baseline response data further to more environmentally friendly products, including Azamax, SeroX, and a *Bacillus thuringiensis* product (Group 11A). Further, other chemical insecticides that have not been tested so far should be examined. These could include abamectin, emamectin benzoate, cyromazine, and an unregistered product, Simodis (Group 30–Isocycloseram). Baseline response data for potential alternative active ingredients should be established before resistance develops. Robust baseline data allows accurate and timely detection of insecticide resistance, providing the groundwork for appropriate actions to conserve susceptibility.
- Spirotetramat (Group 23) has previously been proven to be more effective in controlling insect juvenile stages (Nauen et al. 2008). Consequently spirotetramat was likely ideal for the control of SLM eggs and larvae mining inside the leaf tissue. However, we found heterogeneous resistance to this chemical in this study. Therefore, we recommended that the mechanism of spirotetramat resistance and the fitness cost of spirotetramat-resistant SLM be elucidated. Knowing the mechanism and stability of resistance aids sustainable management if it can be demonstrated spirotetramat resistance reverts to susceptibility.
- It is essential for the industry to undertake routine SLM DNA-based resistance monitoring using the multiplex amplicon sequencing platform developed in this study. This proactive approach will provide industry with early information about changing resistance frequencies. Such knowledge allows for timely and effective actions before gene frequency consequences are seen at the field level. This empowers industry as they stay ahead of any potential resistance issues but also highlights the industry's responsibility to take proactive and responsible measures.
- The genomes of the American SLM, vegetable leaf miner, and other native leaf miner species have not been sequenced in this study. That has prevented the development of DNA-based resistance testing for those species. Whole genome sequencing of these species is required to understand the genetic makeup of these species and facilitate the development of a DNA-based platform for effective resistance management.
- Industry engagement and commitment are key in the fight against insecticide resistance. Therefore, ongoing extension activities, including workshops, field days, demonstration sites, webinars and industry conferences should be facilitated. We envisage this by established industry links in conjunction with Horticulture Innovation Australia. Such activities can play a crucial role in raising the awareness of SLM insecticide resistance and so delay future resistance development.

Refereed scientific publications

Journal article

D. T. Nguyen, Y. Chen, and G. A. Herron. 2024. Insecticide resistance in Australian populations of the serpentine leaf miner *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). Austral Entomology. AEN-6168 (under peer journal review).

Y. Chen, G. A. Herron, J. Webster, and D. T. Nguyen. 2024. Homozygous multiple-insecticide resistance in Australian invasive serpentine leaf miner *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). In preparation, being submitted to the scientific journal *Insects*.

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Intellectual property

No project commercialisation to report.

Project IP was registered and uploaded in Hort Innovation's Delivery Partner Portal along with milestone report 106.

Project data in Appendix A and the manuscript in Appendix B are not for publication at the time of reporting.

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Appendices

Appendix A: Project data tables and figures

Table A1. Details of serpentine leaf miner samples used for bioassays and screened for target-site resistance causing mutations.

№	Sample name	IP condition	Collected location	GPS location	Collection date	Host	Resistance testing method	
							Genomics - № of tested DNA samples	Bioassay - Chemicals tested
1	779_020-00398/1,6	Pre-existing IP	NSW 2250	-33.36680, 151.24021	18/11/2020	Cucumber	2	-
2	740_M20-15983	Pre-existing IP	NSW 2175	-33.83365, 150.85882	19/11/2020	Pumpkin, zucchini, beetroot, radish	3	-
3	746_M20-15989	Pre-existing IP	NSW 2157	-33.60652, 150.99190	19/11/2020	Vegetables	1	-
4	762_M20-16036	Pre-existing IP	NSW 2560	-34.12451, 150.81238	23/11/2020	Runner bean	2	-
5	778_M20-16156	Pre-existing IP	NSW 2622	-35.46909, 149.92253	24/11/2020	Vegetables	1	-
6	808_M20-16339	Pre-existing IP	NSW 2570	-34.08529, 150.66530	25/11/2020	Sticky traps	2	-
7	876_M20-17310	Pre-existing IP	NSW 2318	-32.75242, 151.86899	10/12/2020	Vegetables	3	-
8	Gurner	Pre-existing IP	NSW 2179	-33.91244, 150.81514	17/03/2021	Tomato	10	-
9	Riley-1	Pre-existing IP	NSW 2179	-33.980146, 150.787606	17/03/2021	Shallot	-	-
10	Riley-2	Pre-existing IP	NSW 2179	-33.980146, 150.787606	3/05/2021	Bok choy	15	Imidacloprid, Dimethoate, Chlorantraniliprole, Spirotetramat
11	Riley-3	Pre-existing IP	NSW 2179	-33.980146, 150.787606	3/05/2021	Cos Lettuce	19	-
12	Ross C	Pre-existing IP	NSW 2557	-33.92016, 150.79048	17/08/2021	Cucumber	20	-
13	AusB	Pre-existing IP	NSW 2179	-33.94046, 150.80999	17/08/2021	Broadbean	10	-
14	Kemp CB	Pre-existing IP	NSW 2178	-33.91013, 150.76351	17/08/2021	Cucumber	10	-
15	Kemp C	Pre-existing IP	NSW 2178	-33.888182, 150.779719	17/08/2021	Cucumber	10	Imidacloprid, Dimethoate, Chlorantraniliprole, Spirotetramat Spinetoram

16	Wyreema-2021	Pre-existing IP	Queensland 4352	-27.657108, 151.861549	25/06/2021	Celery	54	Imidacloprid, Dimethoate, Chlorantraniliprole, Spirotetramat, Spinetoram
17	Wyreema-2022	Project IP	Queensland 4352	-27.65572, 151.79925	3/11/2022	Wombok	40	-
18	Aratula	Project IP	Queensland 4309	-27.974936, 152.543096	4/10/2022	Vegetables	32	-
			Total				**234**	

Table A2. Multiamplicon panel and primer sequences for insecticide resistance detection in SLM

Amplicon	Amplicon (bp)	Gene	Primer sequence	Mutation
lmACE1	528	*AChE*	F: GCATTGCATTACACTTTGTGG R: GTGGAGGCTTCATGACAGGT	I129V [a] G227A[a]
lmACE2	170	*AChE*	F: CGTGGATGCCAAGACAATTT R: CATCTTTCACATTGCCAATCA	F331W[a]
KDR1014	213	*VGSC*	F: ACGAACCGAAATTGGACAAG R: GTGCTATGCGGAGAATGGAT	L1014F[b]
KDR918	158	*VGSC*	F: ACGAACCGAAATTGGACAAG R: GTGCTATGCGGAGAATGGAT	M918T[b]
GluCl	164	*GluCl*	F:TTGAGATGTCACTACATTGCTCAC R: GGGAGGCGTAATTGACGAG	A309V[c]
CSH1	193	CSH1	F: GGCGAGGATGGAATAGGTT R: CAGGCGACAACATTCAGAT	I1017F[d]
ACC	150	*ACC*	F: TTCCCTCCGTTTCTAGCACA R: ATACAAACGTCCCGTGCTC	A2083V[e]
COI	549	*COI*	F:CGGAGCTTGAGCTGGAATAG R: GCTCCGGCTAATACTGGTAATG	

The numbering is based on: [a] *Tetronarce californica;* [b]*Musca domestica;* [c]*Drosophila melanogaster;* [d]*Tetranychus urticae;* [e]*Bemisia tabaci*

Table A3. List of insecticide target genes identified

Contig	Target-site gene	Chemical group (Active ingredient)	CDs(bp)	Protein(aa)	GenBank
DN961_c0_g1_i1	Acetylcholinesterase *(AChE*	Group 1A: Carbamates (e.g. methomyl, carbaryl…) and Group 1B: Organophosphates (e.g. chlorpyrifos, dimethoate, trichlorfon…	1971	656	PP481691
DN4884_c0_g1_i3	Gamma-aminobutyric *acid receptor (RDL)*	Group 2B Cyclodienes and Fiproles (e.g. ethiprole, fipronil)	1644	547	PP536544
DN1848_c1_g1_i20	Voltage-gated sodium channel *(VGSC)*	Group 3A: Pyrethroids (e.g. bifenthrin), permethrin, alpha-cypermethrin, zeta-cypermethrin, cyhalothrin…)	6339	2112	PP481692
DN1848_c1_g1_i49	Voltage-gated sodium channel *(VGSC)*		6402	2133	PP481693
DN1848_c1_g1_i53	Voltage-gated sodium channel *(VGSC)*		5403	1800	PP474974
DN2088_c0_g1_i13	Nicotinic acetylcholine receptor subunit *(nAChRβ1)*	Group 4: Neonicotinoids (e.g. Imidacloprid, thiamethoxam, clothianidin...)	1578	525	PP474976
DN2841_c0_g1_i13	Nicotinic acetylcholine receptor Dalpha6 subunit *(nAChRα6)*	Group 5: Spinosyns (e.g. spinetoram, spinosad)	1641	532	PP474975
DN873_c0_g1_i11	GluClalpha *(GluCl)*	Group 6: Avermectins (e.g. abamectin, emamectin benzoate…)	1356	451	PP474978
DN2245_c0_g1_i3	Chitin synthase *1 (CSH1)*	Group 10: Etoxazole, Hexythiazox and Clofentezine	4773	1590	PP474977
DN3210_c0_g1_i21	Ryanodine receptor *(RyR)*	Group 28 : Diamides (chlorantraniliprole, cyclaniliprole, cyantraniliprole …)	15360	5119	PP536545

Table A4. Chemical groups, target sites and mutations detected by multilocus amplicon sequencing and whole genomic sequence

Chemical group (Active ingredient)	Target site gene	Point mutations	Results	Detection method
Group 1A Carbamates (e.g. methomyl, carbaryl…) and Group 1B Organophosphates (e.g. Chlorpyrifos, **Dimethoate**, **Trichlorfon** [(1)])	*AChE*	I129V, G227A	Homozygous resistant	Multilocus amplicon sequencing
		F331W	Homozygous resistant	Whole genome sequencing
Group 2B Phenylpyrazoles (e.g. Ethiprole, Fipronil)	*RDL*	A301S	Homozygous resistant	Whole genome sequencing
Group 3A: Pyrethroids (e.g. **Bifenthrin**[(1)], Permethrin, Alpha-cypermethrin, Zeta-cypermethrin, Cyhalothrin)	*VGSC*	L1014F	Homozygous resistant	Multilocus amplicon sequencing
		M918T	Homozygous resistant	Multilocus amplicon sequencing
Group 4: Neonicotinoids (e.g. **imidacloprid**[(1)])	*nAChRβ1*	R81T	Homozygous susceptible	Whole genome sequencing
Group 5: Spinosyns (e.g. Spinetoram, **Spinosad**[(1)])	*nAChRα6*	G275E	Homozygous susceptible	Whole genome sequencing
Group 6: Avermectins (e.g. **Abamectin**[(1)])	*GluCl*	A309V	Homozygous susceptible	Multilocus amplicon sequencing
Group 10: Etoxazole, Hexythiazox and Clofentezine	*CHS1*	I1017F	Homozygous susceptible	Multilocus amplicon sequencing
Group 23: Tetronic/tetramic acid derivatives (**Spirotetramat**[(1)]**,** Spirodiclofen)	*ACC* [(2)]	A2083V	NA	NA
Group 28 : Diamides (**Chlorantraniliprole**[(1)], Cyclaniliprole**,** **Cyantraniliprole**[(1)])	*RYR*	I4790M	Homozygous resistant	Whole genome sequencing
NA	*COI*	NA	NA	Multilocus amplicon sequencing

Notes:

(1): The active ingredients highlighted in bold are in the list of chemical control options for SLM listed by NSW DPI https://www.dpi.nsw.gov.au/biosecurity/plant/insect-pests-and-plant-diseases/serpentine-leaf-miner/chem-man

(2) PCR assays were troubleshot for this pair of primers, however, they failed to amplify the expected amplicon.

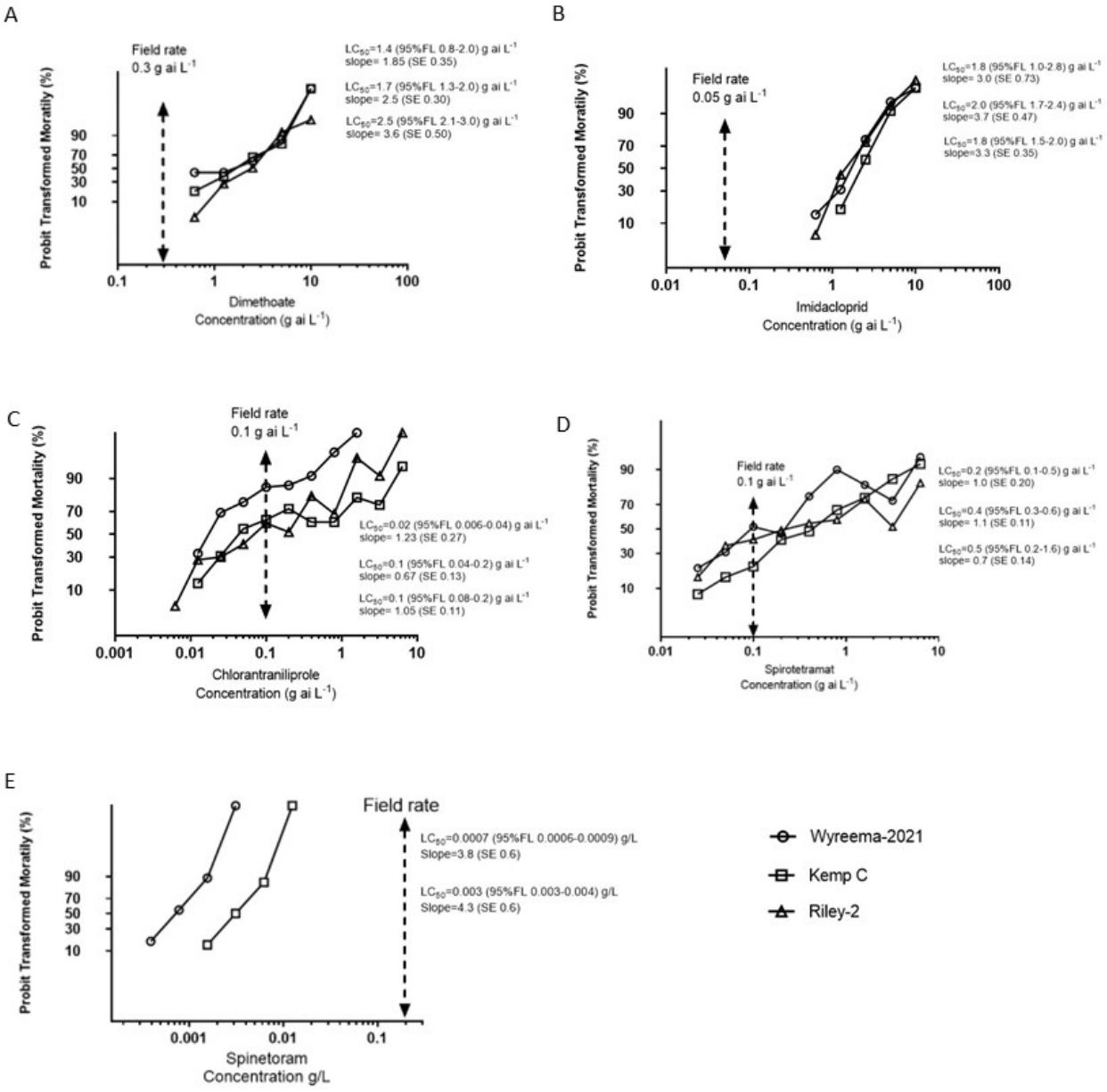

Figure A1. A-E Baseline dose-response for Wyreema-2021, Kemp C and Riley-2 strains of *L. huidobrensis* against: A, Dimethoate; B, Imidacloprid; C, Chlorantraniliprole; D, Spirotetramat; and E, Spinetoram.

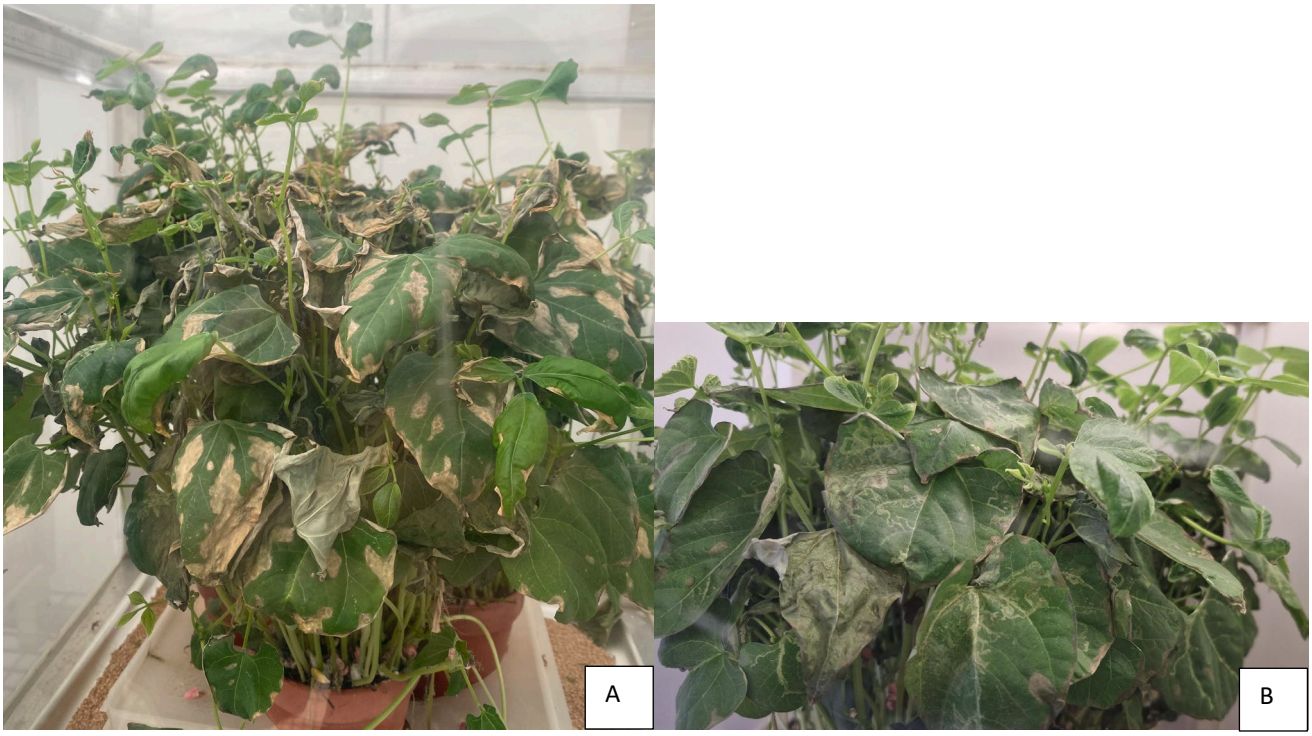

Figure A2. NSW SLM strain Riley-2 survived and successfully produced offspring in potted bean plants with burnt leaves caused by a spray of 4 g L^{-1} dimethoate: A: Treated plants 2 days after spray, B: Treated plants 5-6 days after spray with SLM mining tracks on leaves

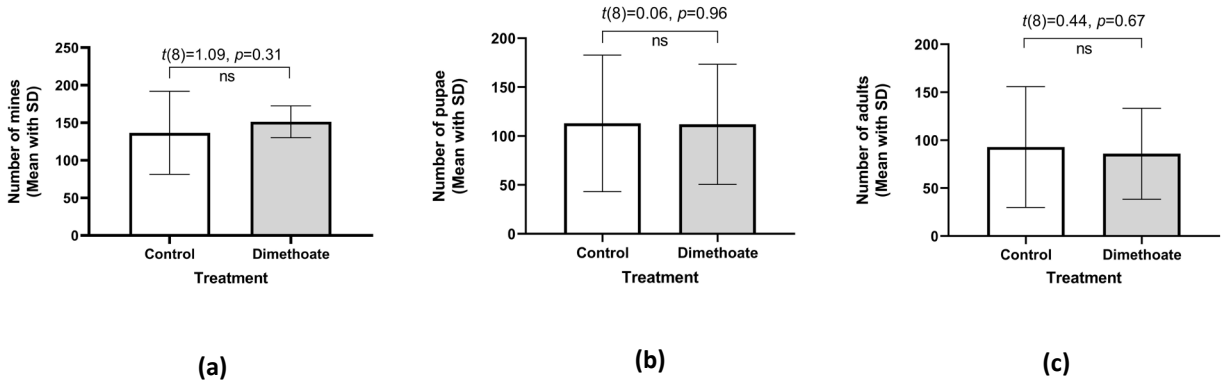

Figure A3. Dimethoate glasshouse trials: effect of dimethoate on the mean number of mines (a), pupae (b), and adults (c) (with associated SD)

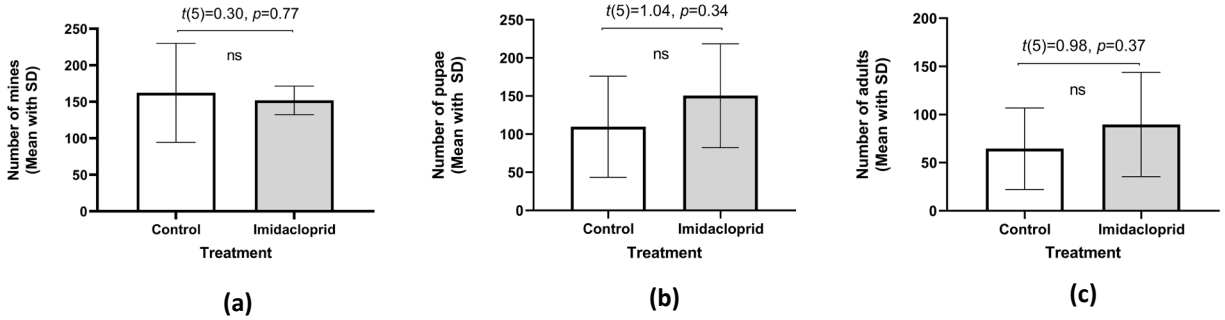

Figure A4. Imidacloprid glasshouse trials: effect of Imidacloprid on the mean number of mines (a), pupae (b), and adults (c) (with associated SD)

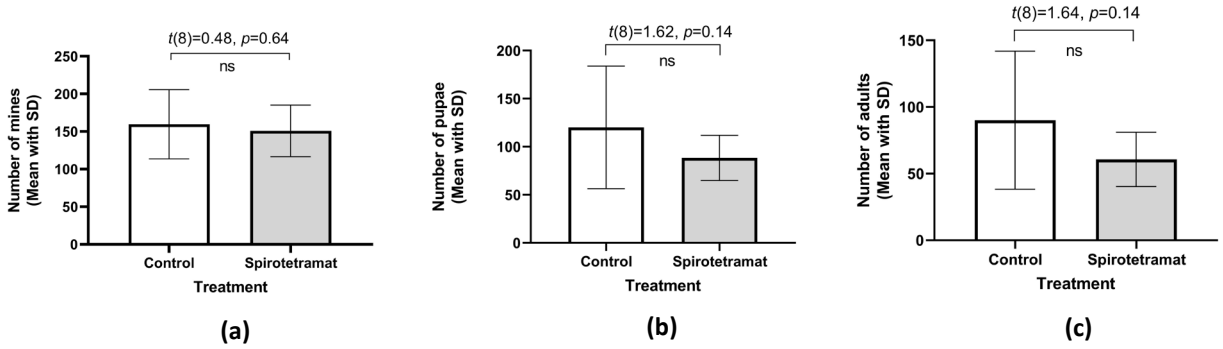

Figure A5. Spirotetramat glasshouse trials: effect of spirotetramat on the mean number of mines (a), pupae (b), and adults (c) (with associated SD)

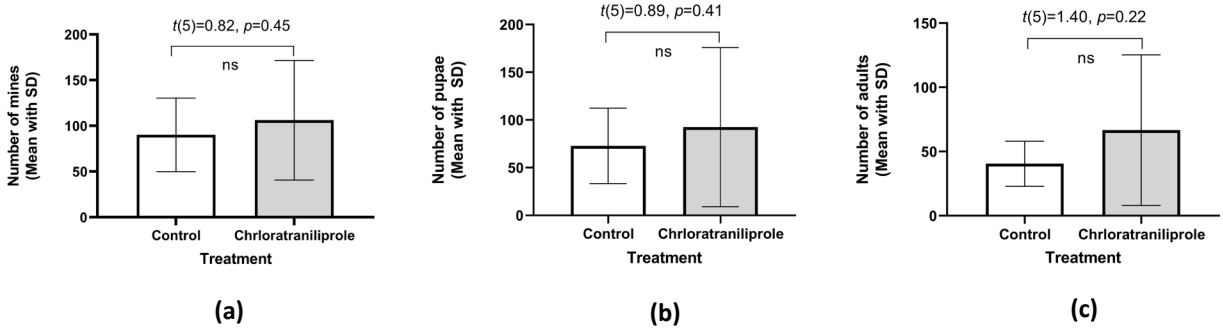

Figure A6. Chlorantraniliprole glasshouse trials: effect of chlorantraniliprole on the mean number of mines (a), pupae (b), and adults (c) (with associated SD)

Appendix C: One-page flyer for project extension activities

Resistance status of serpentine leaf miner to chemicals with minor use permits

AS20002 - Management of insecticide resistance in serpentine leafminer (*Liriomyza huidobrensis*)

Drs Duong Nguyen, Yizhou Chen and Grant Herron
Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries

Active Ingredient		Dimethoate	Imidacloprid	Spirotetramat	Chlorantraniliprole	Cyantraniliprole	Bifenthrin	Fipronil	Spinetoram, Spinosad	Abamectin	Emamectin benzoate	Cyromazine	Diflubenzuron
Mode of action		1B	4A	23	28	28	3A	2B	5	6	6	17	15
Activity		Contact & Systemic	Systemic with translaminar & contact	Systemic with translaminar	Systemic	Systemic	Contact	Contact	Contact with translaminar	Contact with translaminar	Translaminar	Translaminar	Contact, insect growth regulator
Example product		Dimethoate 400	Confidor® 200 SC	Movento® 240SC	Coragen®	Benevia®	TALSTAR® 250 EC	na	Success Neo®, ENTRUST®	Vertimec®	Warlock®	Diptex 150WP	Dimilin W.P. 250
Permit number		PER89184	PER9795	PER88640	PER87631	PER90927	PER9795	na	PER90928	PER81876	PER87563	PER81867	PER90454
Manufacturer's field recommended rate		0.3 g/L	0.05 g/L	0.096 g/L	0.1 g/L	750 mL/ha	2mL/10L	na	400 mL/ha	na	na	na	30-60g/100L
Resistance status tested by NSW DPI Entomology Insecticide Resistance & Genomics	Bioassay: Field rate/ Baseline data	☑ **Resistant** ☑ Dose to control ~100% tested SLM was **33-fold** the field rate recommended by manufacturer ☑ Baseline: **LC_{50}** ranged from **1.4** (95% FL 0.8-2.0) to **2.5** (95% FL 2.1-3.0) g ai L^{-1}	☑ **Resistant** ☑ Dose to control ~100% of tested SLM was **200-fold** the field rate recommended by manufacturer ☑ Baseline data: **LC_{50}** ranged from **1.8** (95% FL 1.0-2.8) to **2.0** (95% FL 1.7-2.4) g ai L^{-1}	☑ **Resistant with varied response** ☑ The highest possible dose that controlled ~ 84% of tested SLM was ~ **64-fold** the field rate recommended by manufacturer ☑ Baseline data: **LC_{50}** ranged from **0.2** (95% FL 0.1-0.5) to **0.5** (95% FL 0.2-1.6) g ai L^{-1}	☑ **Resistant with varied response** ☑ The highest possible dose that controlled ~ 94% of tested SLM was ~ **64-fold** the field rate recommended by manufacturer ☑ Baseline data: **LC_{50}** ranged from **0.02** (95%FL 0.006-0.04) to **0.1** (95%FL 0.04-0.2) g ai L^{-1}	☑ Yet to investigate ☑ Not contracted by AS20002	☑ Yet to investigate ☑ Not contracted by AS20002	☑ Yet to investigate ☑ Not contracted by AS20002	☑ **Susceptible** ☑ The field rate recommended by manufacturer works well controlling 100% tested populations ☑ Baseline data: **LC_{50}** ranged from **0.0007** (95%FL 0.0006-0.0009) to **0.003** (95%FL 0.003-0.004) g ai L^{-1}	☑ Yet to investigate ☑ Not contracted by AS20002	☑ Yet to investigate ☑ Not contracted by AS20002	☑ Yet to investigate ☑ Not contracted by AS20002	☑ Yet to investigate ☑ Not contracted by AS20002
	Molecular	☑ Homozygous resistant mutations detected: I129V, G227A, F331W	☑ No target-site mutation detected ☑ Detoxification yet to investigate	☑ Yet to investigate	☑ A homozygous mutation, I4790M, detected ☑ Yet to investigate the second known mutation, G4946V	☑ A homozygous mutation, I4790M, detected ☑ Yet to investigate the second known mutation, G4946V	☑ Two homozygous mutations, L1014F and M918T detected	☑ A homozygous resistant mutation detected: A301S	☑ Target site mutation was not detected	☑ Yet to investigate	☑ Yet to investigate	☑ Yet to investigate	☑ Yet to investigate

Contact: T 02 4640 6389 M 0452 510 599 E duong.nguyen@dpi.nsw.gov.au