Final Report

Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

Project leader:

John Duff

Delivery partner:

Department of Agriculture and Fisheries Queensland (DAFQ)

Project code:

MT20005

Project:

Management strategy for serpentine leafminer, Liriomyza huidobrensis (MT20005)

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John D. Duff

Delivery partner:

Queensland Department of Agriculture and Fisheries (Project Lead), Cesar Australia, Ausveg, NSW Department of Primary Industries, The University of Melbourne, WA Department of Primary Industries and Regional Development, NT Department of Industry, Tourism and Trade

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Public summary

The project commenced with a focus on Serpentine leafminer (SLM – *Liriomyza huidobrensis*), initially detected in western Sydney, New South Wales, October 2020 and soon after in the Fassifern Valley west of Brisbane, Queensland. The American serpentine leafminer (ASLM – *Liriomyza trifolii*) followed suit arriving in far north QLD, NT and Kununurra WA 2021. With the Vegetable leafminer (VLM – *Liriomyza sativae*) already in Australia since 2015 in far north QLD, Project MT16004 'RD&E program for control, eradication and preparedness for vegetable leafminer'. These introduced *Liriomyza* leafminers can significantly impact a wide range of commercial crops (vegetables, broadacre, ornamentals and non-commercial hosts); and categorised as high priority pests and considered a serious threat to these industries.

This multi-industry, QLD DAF led collaboration incorporates organisations with recent, relevant R&D into leafminers as well as field scientists located in affected regions. The multidisciplinary team developed and delivered specific *Liriomyza* information with emphasis on the species found within the regions and facilitated a targeted communication program, which is critical if susceptible horticultural industries are to better understand and manage these pests.

Key outputs have been a refinement for the eDNA assays for *L. sativae, L. trifolii* and *L. huidobrensis* (Sooda et al. 2017) and *L. brassicae* (Pirtle et al. 2021). This test can now determine the presence of 2 additional leafminers, *L bryoniae, L chinensis*. Real-time qPCR assays were compared with a small portable qPCR machine for use in the field. This was done for *L. brassicae* and *L. huidobrensis* in the field with results for flies and larvae comparable to those achieved in the laboratory. This project developed 2 LAMP (Loop-Mediated Isothermal Amplification) tests that are highly sensitive and specific for serpentine leafminer identification using Genie III machine. For direct visualization of the test result in field, this project evaluated a colorimetric method for identification.

The grower guide "Monitoring for serpentine leafminer in Australia", provides information for conducting effective and efficient monitoring of these pests in Australia. Four grower guides (Vegetable, Potatoes, Melons and Onions) address the differences in management considerations that will occur between different cropping systems. An Industry Management Plan (IMP) includes many aspects of managing these pests by the industry, engaging with relevant stakeholders to ensure effective business and trade continuity.

Extension efforts have been broad ranging, delivering 16 workshops, 3 webinars, 2 field days, 4 grower and agronomist meetings, 2 conferences, 2 melon roadshows and visiting 46 farms. The key outcomes of this project have increased awareness and significance of these 3 leafminer pests, their host range, how they are suited to various regions, what to look out for, and the significance of beneficial insects in managing them and how to look after these beneficial insects by selectively using insecticides.

This project has developed a number of documents which will are available on the Ausveg website [MT20005 – Management Strategy for serpentine leafminer (Liriomyza huidobrensis) | AUSVEG] as well as 2 publications on the spread of these leafminers and using LAMP as a diagnostic tool to help identify at least SLM.

Keywords

Serpentine Leafminer, Liriomyza huidobrensis, American Serpentine Leafminer, Liriomyza trifolii, Vegetable Leafminer, Liriomyza sativa, Leafminer, Integrated Pest Management, parasitoid, vegetable, potato, onion, melon, monitoring

Introduction

The Serpentine leafminer SLM (*Liriomyza huidobrensis*; Agromyzidae) is an exotic leafminer that is highly polyphagous, affecting a wide range of horticultural industries, particularly vegetable, melon, and nursery. This serious plant pest was initially discovered in western Sydney in a peri-urban area where it caused serious damage on one property. Delimiting surveillance for SLM found it in several regional areas of NSW (e.g., Dubbo, Orange) and one location in south-east Queensland (Fassifern Valley).

The American Serpentine leafminer ASLM (*Liriomyza trifolii*) was subsequently detected in multiple locations in Torres Strait, in Far North Queensland, and in Kununurra, Western Australia, and has now been confirmed near Bamaga in the Northern Peninsula Area of Cape York (QLD), in Broome (WA), and in Darwin and Katherine (NT) following broader surveillance activities.

This brings the tally to 3 recently introduced *Liriomyza* leafminer flies into Australia since the start of MT16004 RD&E program for control, eradication and preparedness for vegetable leafminer back in 2015. All *Liriomyza* spp. are now considered as not technically feasible to eradicate and have been included as part of the broader management programs delivered by the relevant State and Territories.

Agromyzidae are a well-known family of small black and yellow, morphologically similar flies, whose larvae feed internally on plants, often as leaf and stem miners, reducing photosynthetic capacity of infested plants, which can cause a significant reduction in yield. Several species in this family are highly polyphagous and have become major pests of agriculture and horticulture in many parts of the world. These three *Liriomyza* leafminers detected in Australia have a wide host range, attacking over 200 hosts species. These include many horticultural crops such as brassicas, beans, lettuce, celery, spinach, onions and other alliums, melons, solanaceous crops (especially potatoes), cut flowers etc. They are widespread in North and South America, Asia, Africa, and Europe. They can move large distances through human-induced dispersal, particularly in nursery stock, where unhatched eggs in leaf material and pupae in soil can easily go unnoticed.

Their life cycle can make them difficult to manage, as foliar pesticides can have limited effect on the larvae inside leaf mines or the pupae under the soil. These flies are also known to develop insecticide resistance making them very difficult to control. Incorrect application of insecticides and choice of insecticides can lead to a decline in beneficial insects and a subsequent population explosion of leafminer flies. Integrated pest management (IPM) techniques and in particular beneficial insects are critical for successful control (Ridland et al., 2020).

This project has built on the outcomes of the previous investment, MT16004 'RD&E program for control, eradication, and preparedness for vegetable leafminer', which was varied in 2019 to also focus on Serpentine leafminer (SLM) and American serpentine leafminer (ASLM).

Methodology

This project built on the work of MT16004, critically adapting and developing targeted R&D specifically for SLM and subsequently ASLM in response to the incursions detected in late 2020 and mid 2021 respectively. The following methodology outlines project activities undertaken and how the achieved outcomes have built on those from MT16004. More detailed methodologies for each component of the project can be found in Appendix 1.0

Component 1. Develop an in-field diagnostic test for serpentine leafminer

(Led by Cesar Australia)

Work program:

1. *Improving outcomes for empty leafminer samples:*
2. *Validating in-field test for SLM:*
3. *Application - Confirming host plant and geographic range for SLM:*
4. The NSW DPI DNA barcode facility (led by Dr. David Gopurenko) at Wagga Wagga Agricultural Institute provided two levels of additional research to the diagnostics of SLM:
   A. Parallel development of rapid and low technology in-field molecular diagnostics of SLM using isothermal amplification methods such as RPA or LAMP
   B. Development and validation of the specificity of DNA sequence probes essential for molecular diagnostics of SLM

Variation October 2021
ASLM specimens were collected and sent to both Cesar Australia and NSW DPI for molecular diagnostics work using qPCR and validating the LAMP SLM diagnostics making sure that this tool is specific to SLM and will not be able to register ASLM at the same time.

Component 2. Develop the surveillance protocol for serpentine leafminer

(Led by Cesar Australia and supported by other parties)

Work program:

1. *Practical and standardised monitoring protocols were developed for SLM infected areas. This was then adapted to those regions that had ASLM detections in the NT and northern areas of WA:*
2. *Validating the establishment model and seasonal risk forecasts were developed:*
3. *The online portal now includes ASLM, SLM and VLM with updating seasonal pest forecasts as well as where these pests have recently been detected*:

Component 3. Develop an industry management plan for serpentine leafminer, ensuring that synergies and conflicts with management for fall armyworm (Spodoptera frugiperda) are taken into consideration

(Led by Ausveg and supported by other parties)

Work program:

1. AUSVEG have collected data from researchers and industry to develop the industry management plan. It is currently awaiting formatting.
2. AUSVEG have also been developing four commodity grower guides to address the differences in management considerations that will occur between the different cropping systems.

These will now include ASLM as well as VLM as all three Liriomyza species are currently in Australia.

Component 4 and 8. Management and engagement strategy and associated extension material (workshops face-to-face and/or webinar, factsheets, podcasts) to drive educational material to growers and regional biosecurity and extension agents (VegNET), and to demonstrate in-field diagnostics and surveillance protocols – linking with Hort Innovation Extension and Communications team and industry extension projects.

(Led by Ausveg and supported by other parties)

Work program:

New extension materials was provided for the development of the four grower guides as indicated in Component 3.
Up to 23 workshops across Western Australia, Queensland and the Northern Territory, along with additional one-on-one grower engagements were conducted during the life of this project. AUSVEG will leverage the existing networks within the industry as part of the communication and extension program, in planning for the workshops and will engage with a range of stakeholders including VegNET officers, biosecurity extension officers and personnel, state DPI stakeholders,

NAQS and rangers, agronomists, schools and community gardens on the management of SLM, ASLM, VLM.

The creation of a demonstration site that could show the effect of inappropriate chemical management (i.e such as the imagery found in Chirinos et al. 2017) was initiated but then suffered a severe weather event and was abandoned. A subsequent "Host preference" trial was established in Spring 2023. Extension activities were significantly expanded to including all three leafminer species (*L. huidobrensis*, *L. sativae*, and *L. trifolii*) into the industry management plans, including strategies to mitigate the spread of *L. trifolii* further south.

Component 5. Develop a spread model for serpentine leafminer in Australia using fine-scale spread data, and incorporating this data into seasonal pest-risk forecasts

(Led by Cesar Australia)

Work program:

1. *Validation of the spread model and extension to field management was undertaken*:
2. *Creation of SLM online portal was developed*:

Component 6. Survey parasitoids of serpentine leafminer in affected regions and indicate how beneficial insects can be incorporated into a broader pest management plan

(Led by QDAF and NSWDPI and supported by University of Melbourne & Dr Peter Ridland)

Work program:

1. *Surveys for parasitoids of SLM was undertaken in both NSW and QLD in regions where this pest was detected*:
2. *Identification of parasitoids was carried out by Peter Ridland at the University of Melbourne*:
3. Due to the subsequent introduction of ASLM in the north of the country, additional surveys were carried out in the NT and WA.

Component 7. Crop protection gap analysis, including how controlling serpentine leafminer will fit into current management strategies and resistance. Working with the Hort Innovation Regulatory Affairs - Crop Protection Manager as appropriate

(Led by Qld DAF and NSW DPI)

Work program:

As this project progressed, a crop protection gap analysis was conducted to identify where additional R&D support may be required. Some initial areas where gaps should be identified included adequate coverage of affected crops with appropriate chemical permits; information necessary for area wide management approaches that account for all pests and chemical uses in a system to preserve beneficial insects; availability of economic thresholds and monitoring protocols for population size estimation.

Results and discussion

Component 1. Develop an in-field diagnostic test for serpentine leafminer

Improving outcomes for empty leaf mine samples (Appendix 1.1)
The project utilised existing publicly available qPCR assays developed for identification of *L. sativae, L. trifolii* and *L. huidobrensis* (Sooda et al., 2017) and L. brassicae (Pirtle et al., 2021) and developed additional qPCR assays for *Liriomyza bryoniae* and *Liriomyza chinensis* and a second more specific assay for *L. huidobrensis*. All six assays were evaluated for their specificity against non-target leaf mine species as well as testing the sensitivity against target leaf mine species calculating the limit of detection (LOD) and limit of quantification (LOQ) for each. Two triplex qPCR assays were able to identify six Liriomyza species, with one assay capable of detecting *L. trifolii, L. huidobrensis*, and *L. sativae*, and the other detecting *Liriomyza brassicae, L. bryoniae,* and *L. chinensis*. Additionally, a control assay was integrated to evaluate PCR inhibition.

Quantitative Polymerase Chain Reaction (qPCR) assays were used to detect and quantify DNA from flies, larvae, and empty leaf mines excised from leaves in both laboratory and field settings. In the laboratory, a modified Chelex extraction protocol was used, which proved to be effective in detecting DNA from all sample types. In-field tests of this assay were conducted using a small portable qPCR machine the Franklin® Real-Time PCR Thermocycler (Biomeme) for two species readily available in the field, *L. brassicae* and *L. huidobrensis*, with results comparable to those achieved in the laboratory using a LightCycler 480 II Real-Time PCR Thermocycler (Roche). Similar qPCR efficiencies were found for the Biomeme and LightCycler (94% and 96%, respectively) and similar cycle threshold values for larvae samples extracted using a simple in-field protocol (ct = 22.4 and 24.3, respectively).

However, in-field DNA extractions from empty leaf mines continue to present a challenge, with a lower quantity of DNA and a higher prevalence of inhibitors, despite trialling numerous extraction protocols. A trade-off was identified between processing sufficient material and effectively releasing DNA from the mines without introducing PCR inhibitors from the plant material. Large amounts of leaf mine material resulted in inhibition, while small amounts of leaf mine tissue caused a loss of sensitivity to detect DNA. Despite using polymerases designed for direct extraction of plant material, there was a limit to how much material could be processed.   Alternate quick field extraction protocols were tested (e.g. QuickExtract Plant DNA Extraction Solution) but this did not provide the sensitivity of the laboratory methods.

The qPCR assays validated herein are of considerable utility given they can be used in a high throughput format within a laboratory setting such as the 384 PCR well LightCycler 480 II (Roche) with 3 species markers per sample well (1152 species assays per run). While the same assays can be utilised in-field with the Franklin® Real-Time PCR Thermocycler (Biomeme) allowing for 9 samples and up to 3 species markers per sample well (27 species assays per run).

Validating in-field test for SLM
Within days of being notified of serpentine leafminer being located in Victoria, samples were collected and tested of celery from supermarkets in Melbourne that exhibited empty leaf mines. These mines were identified as belonging to serpentine leafminer, highlighting the effectiveness of the assay for *L. huidobrensis* on empty leaf mines. Cesar also processed empty leaf mine samples from Cape York Peninsula which were identified as American serpentine leafminer, as well as samples received from the Northern territory which were also identified as American serpentine leafminer. This highlights the effectiveness of this multispecies assay which detects *L. trifolii*, and also confirms the specificity of the *L. huidobrensis* assay. To further improve processing efficiency, Cesar combined their assays into two triplex qPCR assays, one independently detecting the three biosecurity threats (*L. trifolii, L. huidobrensis* and *L. sativae*) one detecting three other species (*L. brassicae, L. bryoniae* and *L. chinensis*), with a further control assay to test for PCR inhibition.

Application – confirming host plant and geographic range for SLM. (Appendix 1.1)
The qPCR multi-species assay was validated against a range of endemic and exotic leafminer species, including collections from different locations (Appendix 1.2). Species screened included; *L. brassicae, L. chenopodii, L. chinensis, L. katoi, L. huidobrensis, L. sativae, L. trifolii, L. bryoniae, L. yasumatsui, Calycomyza humeralis, Cerodontha milleri, Drosophila melanogaster, Ophiomyia alysicarpi, O. solanicola, Phytomyza plantaginis, P. syngenesiae* and *Scaptomyza flava*. The assays showed good specificity for the provided specimens. All samples that did not amplify for one of the six species specific tests were checked with sequencing. Using these assays, 378 leaf miner flies, larvae, pupae, or empty mine samples collected from across Australia and globally were screened. With CO1 sequencing of a subset of 148 of these being undertaken to confirm species identity and primer specificity.

Other activities: *Testing service (Appendix 1.2)*
Cesar Australia offered a testing service to Victorian growers, where a protocol for sampling was developed and distributed to relevant stakeholders. Samples of leaf mines received were screened through the multi-species assays as a free service to growers. Samples were received from three growers in Victoria 28/10/22, 16/11/22 and the 7/12/22 and were able to report results to growers within 3 days of sample receipt. All samples were negative for the assayed species (*L. trifolii, L. huidobrensis, L. sativae, L. brassicae, L. bryoniae* and *L. chinensis*).

The NSW DPI DNA barcode facility (led by Dr. David Gopurenko) at Wagga Wagga Agricultural Institute provided two levels of additional research to this project by:
Developing two LAMP (Loop-Mediated Isothermal Amplification) tests that are highly sensitive and specific for serpentine leafminer identification using Genie III machine. For direct visualization of the test result in field without expensive Genie machine, they evaluated and subsequently shifted from the lateral flow strip method to a more efficient and secure colorimetric method. See Appendix 1.3 for paper on this topic which has just been accepted for publication.

The lateral flow strip (LFS) method requires the LAMP primers to be labelled by two antigens. The modifications for this conversion requires substantial time for development, optimising and validations of an established LAMP protocol. Most crucially, this method mandates the opening of test microtubes to transfer natant or insert a flow strip into the endpoint LAMP product. This raises the very high risk of cross-contamination from positive samples and subsequent false-positive results. Additionally, modification of LAMP primers for lateral flow detection generally results in longer processing time during isothermal amplification and additional time for the subsequent handling steps required for transfer of LAMP products onto and through the LFS. Finally, modification of the test for LFS incurs a greater per sample unit cost.

By contrast, the colorimetric method (Figure 1) offers substantial benefits that address the contamination issues and limitations of the LFS approach. The designed LAMP primer sets can be directly used for colorimetric method without modification to an established protocol, or subsequent needs for additional optimisation and validations required for assessing reliability of the antigen labelling. Critically, the visual interpretation of end-product results without disturbing the microtube eliminates the risk of cross-contamination that is likely to occur through LFS use. Colorimetric testing is also a faster procedure, eliminating the need for additional waiting times typically associated with antigen based LFS detection of LAMP results. Therefore, the decision to transition from the lateral flow strip method to the colorimetric method is well-justified. It enhances the LAMP test's efficacy (Figure 2) and reliability ultimately contributing to more robust and accurate identification of the serpentine leafminer in field."

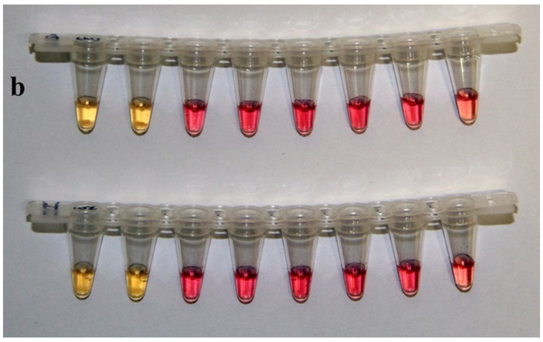
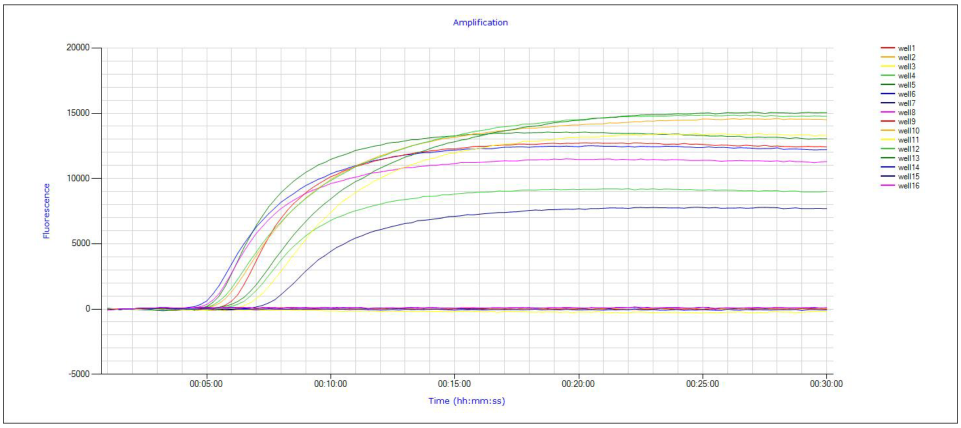


Figure 1. LAMP colorimetric assay Specificity tests CAD (top) & COI (below)
Left to right: *L. huidobrensis*, *L. huidobrensis*,(yellow colour) *Scaptomyza australis*, Agromyzidae sp. indet., *Calycomyza lantanae*, *L. brassicae*, *L. trifolii*, no-template -neg control (pink colour). Image: Xiaocheng Zhu, NSW DPI



Well 1 +ve control LV
Well 2 Okra
Well 3 Zucchini
Well 4 Weeds
Well 5 Okra
Well 6 -ve control
Well 7 Blank
Well 8 Blank
Well 9 +ve control Bundaberg
Well 10 Watermelon pupae
Well 11 Potato pupae
Well 12 Brassica weed
Well 13 Cucumber
Well 14 -ve control
Well 15 Blank
Well 16 Blank

Figure 2. LAMP amplification graph showing positive reactions for SLM on various crops from the Lockyer Valley and Bundaberg regions, November 2023. The straight lines are the -ve controls and Blank wells.

Component 2. Develop the surveillance protocol for serpentine leafminer

Develop practical and standardised monitoring protocols for SLM infected areas:

Cesar researchers lead the development of a surveillance protocol for leafminer and parasitoid surveys and conducted a review of overseas monitoring techniques. The leafminer and parasitoid survey protocol was used by the project team to collect data for three purposes: 1) recording seasonal and regional presence of parasitoid wasps communities to identify biocontrol agents and support the validation of a parasitoid forecast tool; 2) recording seasonal and regional presence of serpentine leafminer and American serpentine leafminer, to support the validation of the seasonal leafminer forecast tool to aid in pest management; and 3) recording the progression of new locations affected by serpentine leafminer and/or American serpentine leafminer. To ensure standardization of data collected between all project partners, standard data collection forms were created, as well as a communal spreadsheet where data is collected and available for use by the predictive modelling team. This surveillance protocol was tested in several sites in QLD and revised accordingly.

The review of overseas monitoring techniques included work on early detection and use of economic thresholds to manage leafminers. A summary of the scope of this short review is included as Appendix 2.1. The review was distributed to all project partners to support surveillance work, as well as extension activities.

Cesar researchers interviewed nine agronomists and growers representing the Fassifern/Lockyer Valleys and the peri-urban Sydney region. The results of these interviews were compiled and provided as a report to project partners, including recommendations for extension activities that could be used to fill some immediate knowledge gaps reported by the interviewees, including: 1) collecting more high resolution imagery of stippling damage and parasitoid species most important in leafminer control for each region; and 2) creating a simple table that includes up to date information on (i) chemicals currently registered for use, (ii) efficacy against serpentine leafminer, and (iii) toxicity to beneficial parasitoids. Notable insights gleaned from this small benchmarking exercise can be found in Appendix 2.3.

Using knowledge gained from the monitoring review and the interviews of agronomists and growers, a concise and illustrated monitoring guide was developed, with the goal of supporting early management of serpentine leafminer (Appendix 2.2). The guide was provided to AUSVEG, QDAF and NSW DPI as a resource for the grower workshops.

High resolution looking at certain diagnostic characteristics between species of *Liriomyza* may be of some help to agronomists. As seen below between SLM and ASLM, there are 2 diagnostic characteristics that could help to separate these 2 species. Others *Liriomyza* species may have similar characteristics so care needs to be taken if looking at external characteristics rather than using a molecular diagnosis.

Image 1. Serpentine leafminer has dark behind the eyes and dark striations on the Femurs. The AMSL has yellow Femurs and yellow behind the eyes.

Validating the establishment model and seasonal risk forecasts:
Building upon modelling outputs from the previous MT16004 project, this project finalized spread models for serpentine leafminer, American serpentine leafminer and vegetable leafminer. This work identified the establishment potential of all three species across Australia. Global distribution data spanning 42 countries was compiled and used to validate the process-based model of establishment potential based on intrinsic population growth rates of each species.

The modelling approach employed, successfully captured the international distribution of the serpentine leafminer, American serpentine leafminer and vegetable leafminer based on environmental variables and predicted the high suitability of non-occupied ranges in Australia. The largely unfilled climatic niche available to these pests demonstrates the early stages of their Australian invasions and highlights locations where vegetable production regions are at particular risk. In addition to Australia, the results highlight many regions globally where serpentine leafminer, American serpentine leafminer and vegetable leafminer have the potential to spread in the future. Countries such as large parts of South America, central Africa, the Pacific and parts of Europe.

In addition to the three leafminer species, we also modelled two cosmopolitan parasitoid wasps known to provide control in both field and glasshouse settings, *Diglyphus isaea* (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae). We found that within Australia, *D. isaea* and *H. varicornis* are predicted to have a large spatial and seasonal overlap with each *Liriomyza* species (Figure 3) and thus are expected to influence the future spread of these pests and play an important role in local pest management programs.

Our work was written up into a scientific manuscript and published in *Austral Entomology* (Appendix 2.4).

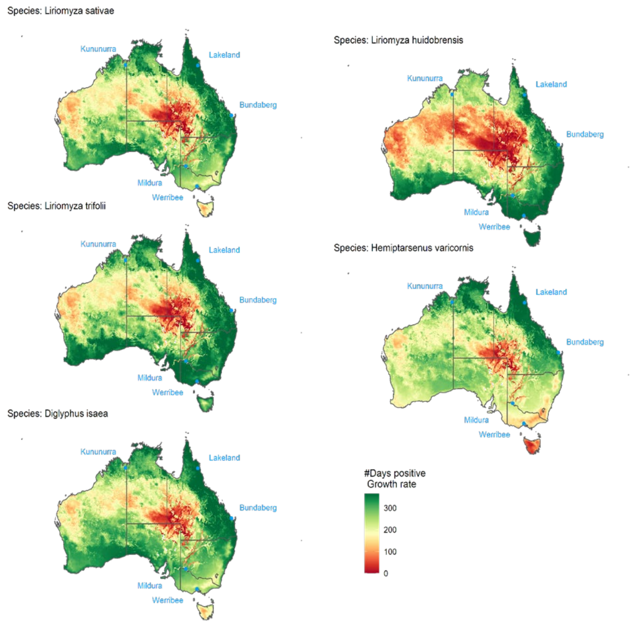


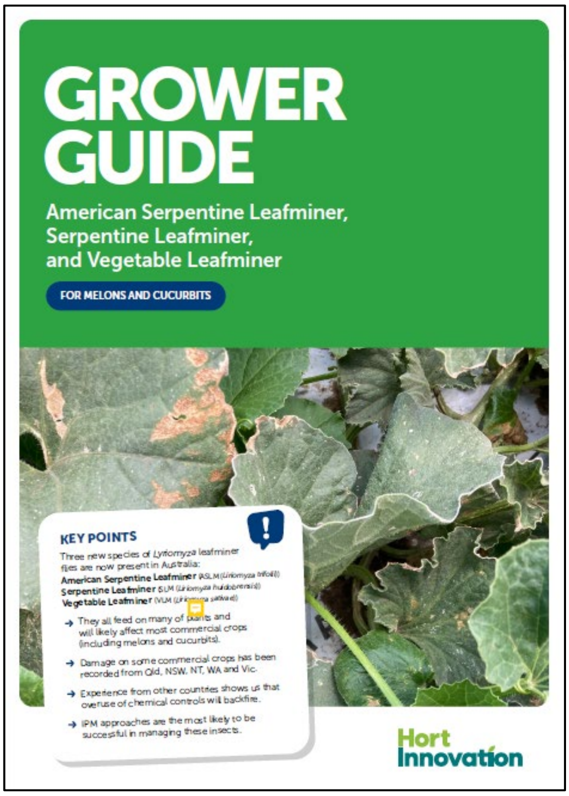
Figure 3. Climatic suitability for vegetable leafminer, serpentine leafminer, American serpentine leafminer, *D. isaea* and *H. varicornis* across Australia*,* depicted as the number of days during which positive growth rates are predicted to be achievable using climate data from 2017. Image source: (Maino et al., 2023) https://doi.org/10.1111/aen.12632

Creation of SLM online portal with updating seasonal pest forecasts:
Refer to component 5.

Component 3. Develop an industry management plan for serpentine leafminer, ensuring that synergies and conflicts with management for fall armyworm (*Spodoptera frugiperda*) are taken into consideration

AUSVEG are developing an industry management plan which will encompass all 3 *Liriomyza* leafminers, ASLM, SLM and VLM. This will be a live document in line with other previously developed documents for tomato potato psyllid (TPP) and Cucumber green mottle mosaic virus (CGMMV). It will include many aspects of managing the pest by the industry, engaging with relevant stakeholders to ensure effective management of the pest and ensure business and trade continuity. It will serve as a guide to each of the contributing industries on managing the impacts of *Liriomyza* leafminers on their industries. It will focus on; 1. Introduction to the Management Plan, 2. Pest Characteristics, 3. Managing the Leafminers in Australia, 4. Surveillance and Monitoring, 5. On farm Management, 6. Trade Implications, 7. Roles and Responsibilities, 8. Gaps in Available Research and Knowledge, and 9. Potential future Research Areas. Due to the complexity of this document, it is only partially complete. All areas are populated with information and will be formatted into a usable document in the new year.

AUSVEG has been developing four commodity grower guides to address the differences in management considerations that occur between different cropping systems. These will include vegetables, potatoes, melons and onions. Available overseas management learnings will be used to guide the appropriate scopes of each guide developed. The regional preparedness plans developed within MT16004 and the enterprise management plans developed under the transition to management phase for the TPP incursion response will provide a basis to develop these crop focused industry grower guides. Because different cropping systems would be able to withstand varying levels of infestation and damage before an economic threshold is reached and action is required to manage the pest, these grower guides will try and reflect these differences. These guides will include ASLM, SLM as well as VLM as all three *Liriomyza* species are now present in Australia. These also are only partially complete with the grower guide for melons and cucurbits nearing completion. Once this grower guide is completed the other 3 will quickly follow as the majority of information will remain the same with the images changing and the differences in management considerations for each commodity being the only changes needed. The latest draft of the below guide can be found in Appendix 3.1.



Component 4 and 8. Management and engagement strategy and associated extension material (workshops face-to-face and/or webinar, factsheets, podcasts) to drive educational material to growers and regional biosecurity and extension agents (VegNET), and to demonstrate in-field diagnostics and surveillance protocols – linking with Hort Innovation Extension and Communications team and industry extension projects.

This project, MT200005, was a biosecurity exotic leafminer incursion response project and aligned closely with the AUSVEG biosecurity program across all states and vegetable commodities and followed the leafminer preparedness project MT16004.

The workplan for this part of the project called for workshops, or appropriate extension activities such as farm visits, in NT, Nth Qld and Nth WA. The team leader from Qld DAF, had conducted a workshop for agronomists in the Granite Belt in December of 2022 and this became a blueprint of the workshops to be rolled out across the North. A typical running sheet for the workshops can be found at the end of Appendix 4.1. Advice and engagement were sought from members of the project team across Australia, local farming associations, regional VegNET officers, relevant State, Territory and Federal government agencies and key local growers.

The workshops incorporated a Monitoring and Evaluation (M&E) Survey for growers and a separate but closely aligned survey for industry participants to gauge the awareness and use of current resources and a set of discussion questions to investigate attitudes and intentions for practice change. Copies of these instruments can be found in the M&E report (Appendix 4.4). Additional evaluation responses can be found in Appendix 4.2 and 4.3.
During the life of this project, there were 10 different types of extension activities held across Australia from field days, farm visits, webinars to workshops. A breakdown of those conducted during the life of this project are included as a summary in the table below. A more detailed list of activities can be found in Appendix 4.1

Table 1 Total number of events organised and participated in as part of MT20005.

Event	Number of events	Attendance
Growers expo	1	45+
Field day	2	600+
Field walks	3	25
Webinars	3	56
Farm visits	46	68

Market visits	3	3
Grower meeting	1	12
Workshops	16	227
Agronomist breakfast & meetings	3	26
Melon roadshow	2	Ayr/Katherine
Conference	2	Adelaide/Ballarat

Investing in ongoing extension activities in IPM systems is needed and should be imbedded in programs such as VegNET and other horticulture industry extension and engagement programs. Funding bodies often remark this has all been done before but there are always new growers and new communities of growers, such as migrants from sub-Sahara Africa we are now seeing in Northern Australia. Even existing established IPM systems face changing pressures and there are new tools and methodologies being released constantly that need to be incorporated.

Serpentine leafminer demonstration site

Image 1. Trial site after flood waters swept through it, Feb 2022. Drone imagery.

A demonstration site was planned in 2022 at the start of autumn to coincide with the anticipated build-up of SLM in the area of the Gatton Research Facility. Unfortunately this also coincides with an extremely large rain event that caused severe flooding and the washing away of this demonstration site, which was subsequently abandoned.

It wasn't until late winter/early spring 2023 that another trial was conducted looking at host preference of SLM. Twenty one different vegetable crops that can be grown in the Lockyer valley were planted as part of an AgTech Show case field day event held at the Gatton Research Facility. Some of these crops are known hosts and others that are supposedly hosts of SLM. Growers in the region had been experiencing leafminer activity, especially in crops such as potatoes and onions and some of the leafy vegetables. Planting dates were staggered to try and get most crops close to maturity for the grower Agtech show case days on the 1st and 2nd November. First crops were planted on the 28th August and final ones planted the 3rd October. This was a time when we felt leafminer should be an issue for the crops planted in this trial. Assessment of leafminer activity started once all the crops were planted (Figures 4 and 5).

Image 2. Replicated host preference trial using 21 vegetable crops at the Gatton Research Facility, October 2023. Drone imagery.

The feeding preference of the serpentine leafminer (SLM), *Liriomyza huidobrensis*, was evaluated in three replications using eighteen vegetable crops in the field. Depending on their development, the various vegetable hosts were planted at different times. The number of plants with leaf mines per plot and the number of leaves with mines on selected plants at the early (1st assessment) and late vegetative (2nd assessment) stages were counted, converted to percentage damage, and graphed to determine host choice.

On this trial, the responses of the different vegetable crops to serpentine leafminer damage differed. Wombok shows a hundred percent of plants with mines per plot among the several hosts examined during the first assessment. Mines were also found in almost all of the plants in celery and cabbage plots, with mines present in 98 and 90.95% of the plants, respectively. In terms of the amount of mined leaves per plant, celery had 68.43 percent of the leaves damaged by SLM, followed by beetroot, which had more than 55 percent of the leaves with mines. Interestingly, no mines were found on the leaves of carrot. During the assessment, the majority of the leaves with mines were located on the lower portion of the plant which may imply that at the early vegetative stage, SLM preferably feed on fully developed and expanded leaves for nutrition or protection from predation or parasitism. Except for potato, where all of the plants within each plot had SLM damage and more than 50% of the leaves had mines, the number of plants and leaves with mines decreased following the second assessment. The reduction in the percentage of plants and leaves damaged by SLM may be attributed to the hot weather conditions prior to and during the week of second assessment, as well as the senescing lower leaves with mines that were not counted. The temperature recorded during the second assessment ranges from 26^0C – 37^0C which may have an impact on SLM activity in the field. The favourable temperature for leaf miner activity ranges from 20 – 25^0C.

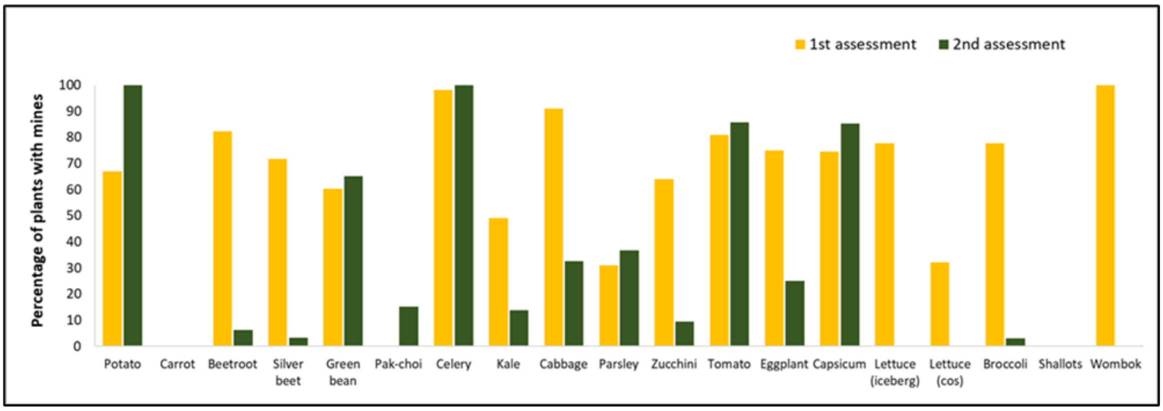


Figure 4. Percentage of plants mined by the serpentine leaf miner on various crops assessed at early and late vegetative stages. Gatton Research Facility, August – November, 2023.

The recent findings suggest that host plants at early stage of the development can be attacked by the serpentine leaf miner with strong preference on potato, celery, and wombok. The SLM may also have an intermediate preference for zucchini, beetroot, silver beet, and broccoli but may not significantly affecting carrot and parsley. In addition, the number of plants with mines may not also directly indicate the severity of damage, but rather the number of mined leaves per plant. However, further trial needs to be undertaken especially during the season when SLM are more active to validate its host plant preference.

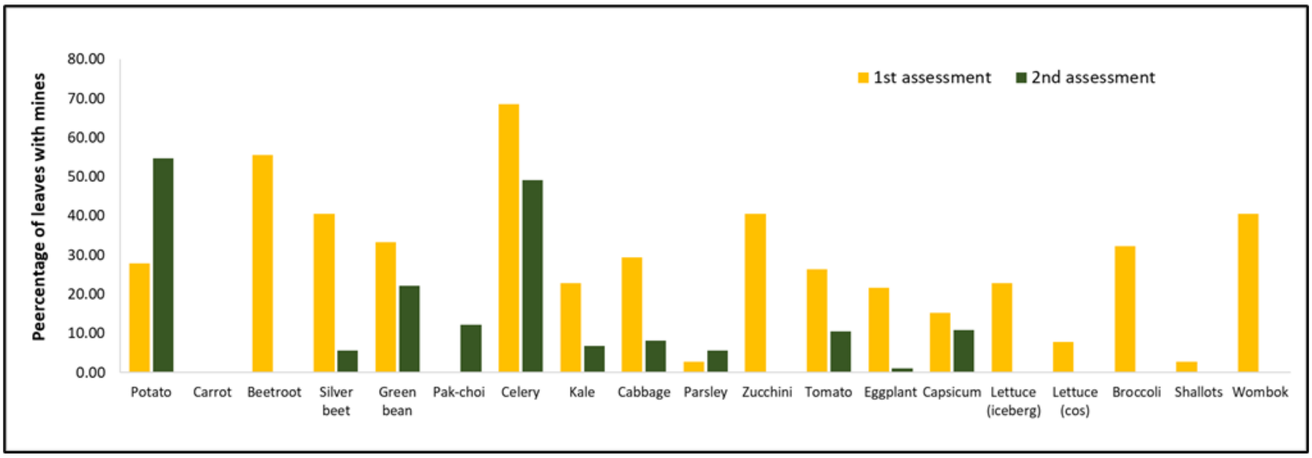


Figure 5. Percentage of leaves with mines by the serpentine leaf miner on each plant of various crops examined during the early and late vegetative stages. Gatton Research Facility, August – November, 2023.

Component 5. Develop a spread model for serpentine leafminer in Australia using fine-scale spread data, and incorporating this data into seasonal pest-risk forecasts

The main objective of the modelling tool is to better capture the link between the seasonal activity of Serpentine leafminer, American serpentine leafminer and Vegetable leafminer and the seasonal activity of their key parasitoids so that growers and advisers can better understand and manage the population dynamics of the pest and its parasitoids. These processes vary across region and season so an approach that considers this spatial variation in conditions is necessary to provide insights that are timely and regionally relevant to affected plant industries. The diagram of the project described by Figure 6 consists of three steps:

1. Mapping collected data within a web-app (using R shiny web-app).
2. Prediction of leafminers and parasitoid populations using model described in 3 (using Julia libraries: GrowthMap.jl, DynamicGrids.jl and Dispersal.jl).
3. Connecting prediction with collected data on the web-app (using R shiny web-app).

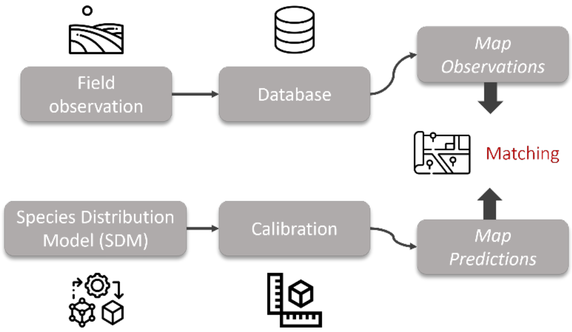


Figure 6 - Project diagram connecting Field Observations to Species Distribution Model (SDM).

Cesar Australia developed a continent-wide dispersal (spread) model for serpentine leafminer, American serpentine leafminer and vegetable leafminer. This work builds upon modelling packages Cesar earlier developed (Schouten et al., 2022) and as summarised in Appendix 5.1. These models are yet to be validated due to the paucity of high-resolution spread data from the field post the recent incursions of the serpentine leafminer and American serpentine leafminer into Australia. However, the spread models have been extended to the two parasitoids, *D. isaea* and *H. varicornis*.
In parallel to the modelling, we created a web application to disseminate project results and offer an easy-to-use mechanism for project partners to produce regionally pertinent visualizations in support of extension activities (e.g. workshops). Initially, Cesar employed R Shiny app technology, but later transitioned to a platform (Flask-VueJs) that allowed us to incorporate more sophisticated features into the tool, such as running simulations directly within the app. While not initially tailored for growers and advisors, the platform was designed with the potential for further development to cater to this end-user scenario. The Flask-VueJs app contains: 1) A mapping interface that plots the field survey data; 2) a growth day module that simulates the growth potential of SLM, ASLM, VLM, *D. isaea* and *H. varicornis*; and 3) a co-occurrence module whereby the biocontrol potential can be simulated for each pest-parasitoid combination. A screenshot of the growth day module for serpentine leafminer in central NSW in January is shown in Figure 7 below.

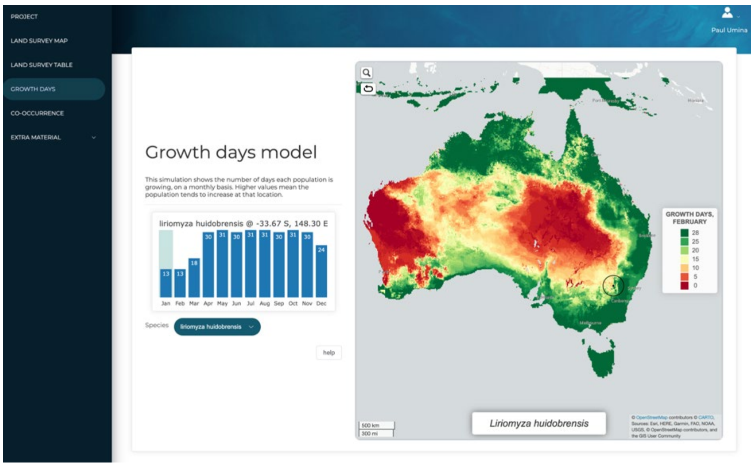


Figure 7: Screenshot of the growth days module in the Flask-VueJs app. In this example, the growth potential of serpentine leafminer in central NSW during the month of January is shown.

The usefulness of this tool to agronomists and growers is debateable. Knowing where and when these leafminers are likely to be around will come about through crop monitoring by agronomists and growers. As long as this information is recorded, season trends can be plotted, which will help individuals with their monitoring at certain times of the year. Showing agronomists and growers distribution maps of where these pests are likely to occur can help with decision making when it comes to growing particular crops known to be hosts of one or all of these leafminers.

Component 6. Survey parasitoids of serpentine leafminer in affected regions and indicate how beneficial insects can be incorporated into a broader pest management plan (Appendix 6.1)

A significant community of parasitoid wasps has been reared in each region from either *Liriomyza huidobrensis* or *L. trifolii*. However, species composition and complexity varied between regions. This probably reflects the fact that the diversity of parasitoid community changes in time as infestations of *Liriomyza* spp. spread. The primary challenge will be to ensure these parasitoids are not disrupted by excessive use of inappropriate insecticides and that reservoirs of parasitoids and non-target agromyzids are identified and managed/conserved effectively.

The parasitoid wasp community reared from *L. trifolii* in NW Western Australia (1,587 parasitoids reared from samples collected between April 2022 and November 2022) was dominated by the idiobiont ectoparasitoids *Zagrammosoma latilineatum* (57%) and *Hemiptarsenus varicornis* (41%). Five other parasitoid species were identified [*Asecodes* sp., *Apotosoma* sp., *Neochrysocharis formosa*, *N. okazakii* (Eulophidae); possible *Gronotoma* sp. (Figitidae, Eucoilinae)] and together with some unidentified Pteromalidae were in very low numbers (2% of all parasitoids). Most of the mined leaves sampled were from the Asteraceae (65%) and Fabaceae (25%). There were no *Opius* spp. (Braconidae) reared from puparia. Within the Asteraceae, 91% of parasitoids were reared from sunflower. Peak numbers of *H. varicornis* and *Z. latilineatum* were recorded in August 2022. In the Northern Territory, the main parasitoids recorded from *L. trifolii* were *H. varicornis*, *Z. latilineatum* and *N. formosa*. An important finding was that *Opius* sp.1 (most likely part of the *O. atricornis* complex) has now been found in low numbers in the NT.

In SE Queensland, 16 identified parasitoid species plus some unidentified eulophids and pteromalids were reared from *L. huidobrensis* (1,279 parasitoids in all). The most abundant parasitoid species were *H. varicornis* (40% of all specimens), *Opius cinerariae* (23%), *N. formosa* (15%), *Asecodes* sp. (6%) and *N. okazakii* (4%). These were the first confirmed Queensland records of *Asecodes* sp., *N. formosa*, *N. okazakii* and *Diglyphus isaea* (all species have been previously recorded from SE Australia attacking agromyzids). The major differences between the parasitoid communities observed in NW WA and SE Qld were the relatively low abundance of *Z. latilineatum* and the high abundance of O*. cinerariae* in SE Qld.

In NSW, six species (*D. isaea, H. varicornis, N. formosa, N. okazakii, O. cinerariae* and possible *Gronotoma* sp.) were reared from *L. huidobrensis* mining faba bean. *Neochrysocharis formosa* was recorded for the first time in NSW and was the most abundant parasitoid.

Specimens of possible *Gronotoma* sp. (Figitidae: Eucoilinae) were reared from *L. huidobrensis* (NSW and Qld) and *L. trifolii* (WA and NT). These are the first Australian records of a Eucoiline parasitizing an agromyzid. Specimens will be sent to USA for examination by a specialist in the family. For a more detailed breakdown of the parasitoids found as a results of this project, check out Appendix 6.1. Investigating how well they parasitise a population of leafminers in the field will involve further research.

Component 7. Crop protection gap analysis, including how controlling Serpentine, American Serpentine and Vegetable leafminers will fit into current management strategies and resistance. Working with the Hort Innovation Regulatory Affairs - Crop Protection Manager as appropriate

As this project progressed, a crop protection gap analysis was conducted to identify where additional R&D support is needed. Some initial areas where gaps have been identified include adequate coverage of affected crops with appropriate chemical permits; information necessary for area wide management approaches that account for all pests and chemical uses in a system to preserve beneficial insects; availability of economic thresholds and monitoring protocols for population size estimation.

When leafminers were first detected on the mainland of Australia, in particular *Liriomyza huidobrensis*, growers initially requested insecticides to manage them. This was particularly the case with leafy vegetables, where the quality of the harvestable product was severely affected. Crops were ploughed in as with certain baby leaf vegetables, or infested leaves trimmed off to try and minimise the incidence of leafminer evidence on the crop such as wombok, silverbeet and celery.

Just because an insecticide is registered for use against leafminers, doesn't mean that it will be effective. Not all leafminers are flies, some are beetles (lantana leaf mining beetle - *Octotoma scabripennis*) while others are moths (citrus leafminer - *Phyllocnistis citrella*; potato/tomato leafminer -*Phthorimaea operculella*). The correct identification is crucial so that the correct insecticide can be applied to give maximum control so long as it is also applied in the correct manner.

Areas that need further work undertaken within Australia include but not limited to:

- Insecticide use both new and old chemistry including biological insecticides and how they affect parasitoids
- Continue to investigate insecticide resistance of introduced *Liriomyza* populations (VLM, SLM, & ASLM)
- Efficacy of different application methods in conjunction with planting densities
- Simple key to morphological characteristics for growers and agronomists
- Augmentative release of parasitoids and understanding their biology
- Endosymbionts to produce female only lines of parasitoids
- Use of Entomopathogens
- Plant extracts/attractants/pheromones
- Economic thresholds of various crops (potatoes, onions, leafy veg, celery)
- Surveillance of seedling nurseries and other cropping areas for both leafminers and parasitoids
- Ongoing awareness of IPM options
- High resolution photos of the leafminers, types of mines they produce, including stippling damage, and the range of parasitoids found to attack these leafminers. A collection of the different leafminers found during this project has been compiled at the end of Appendix 7.1

More detailed information on the gaps in our knowledge can be found in Appendix 7.1

Outputs

Table 3. Output summary

Output	Description	Detail
LAMP (Loop-Mediated Isothermal Amplification) tests using Genie III	Identifying specific target SLM genes and oligo-primers designed to these targets to maximise their specificity to SLM DNA	A rapid and simple DNA extraction method, suitable for LAMP analyses was identified. Two sets of LAMP primers were designed based on in-silico analyses of reported Agromyzid COI sequence accessions and the DNA barcoding efforts. A training protocol is attached Appendix 1.3 Paper under review Appendix 1.4
Monitoring for serpentine leafminer in Australia	Based on the work from MT16004 this guide was put together as a tool for growers, researchers and agronomists.	This guide provides information for conducting effective and efficient monitoring of this pest in Australia. It can also be used for the other 2 *Liriomyza* species now present in Australia. Appendix 2.1
Monitoring to support integrated pest management of *Liriomyza* spp. pests in Australia	A mini-review of global monitoring plans to be used by researchers in developing monitoring guidelines under Australian conditions.	Popular method overseas due to being an easily visual indicator of whether leaf mine damage is caused by an active infestation, or whether the damage is old and thus intervention may be unwarranted; gives accurate population size estimates and can be used with Economic Thresholds.
Interactive online portal containing seasonal SLM and ASLM pest forecasts and visual pest and beneficial population spread outputs to support monitoring	Seasonal SLM pest forecasts will be made available to growers and industry through a user-friendly web interface	Creation of a web application to disseminate project results and offer an easy-to-use mechanism for project partners to produce regionally pertinent visualizations in support of extension activities (e.g. workshops)

and management decisions.		
4 grower guides (Vegetables, Potatoes, Melons and Onions (Partially complete)	Develop four commodity grower guides to address the differences in management considerations that will occur between different cropping systems.	Different cropping systems would be able to withstand varying levels of infestation and damage before an economic threshold is reached and action is required to manage the pest, these grower guides will try and reflect these differences and will focus on seasonality, life cycles, pest impact, integrated pest management, chemical management, monitoring and farm biosecurity
Industry Management Plan (Partially complete)	This document will serve as a guide to each of the contributing industries as a whole on managing the impacts of ASLM, SLM and VLM on their industries.	The national industry management plan will focus on key topics including how industry will work with government to establish movement conditions and treatment options, management options, and surveillance and monitoring guidelines, roles and responsibilities, amongst various other topics such as information on other exotic leafminers to watch out for (*Liriomyza bryoniae* and *Chromatomyia horticola*).
Communication & Engagement Plan	VegNET network - content developed for regional newsletters through the VegNET network and Industry magazine articles.	Webinars, articles for Vegetable Australia and Melons Australia, 4 grower guides, Industry Management Plan
Project Monitoring & Evaluation Plan	Covering such topics as a Program Logic, Key Evaluation Questions, and Performance expectations, data collection and analysis	This was developed as part of milestone 2.
Crop Protection gap analysis	Gap analysis is to identify where additional R&D support may be required.	A document identifying areas of future research has highlighted resistance testing to support insecticide use needs to be expanded as well as looking at the possibilities of newer biological products, it there a more efficient way of application targeting the larvae inside the leaves, augmentative release of parasitoids, the use of plant extracts as attractants/pheromones and detailed work on economic thresholds.

Outcomes

Table 4. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Increased awareness what effect insecticides have on SLM and the parasitoids that use them as hosts.	Vegetables Outcome 1 Strategy 2 and the KPI - Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers Potatoes Outcome 2 Strategy 1 and the KPI Pest and disease management strategies	Growers and agronomists more aware of SLM and ASLM through workshops farm visits etc and the role that beneficial insects play in combatting these pests so that there is less reliance on insecticides to manage their insect pest issues. What impact insecticides have on beneficial insects (parasitoids). Resistance testing of insecticides through the	Grower and Industry M&E survey responses (Appendix 4.2)

	are developed that mitigate crop loss in collaboration with growers Onions	project AS20002 Management of insecticide resistance in serpentine leafminer (Liriomyza huidobrensis)	
Being able to identify the active SLM populations by looking at leaf mines, using monitoring tools and rearing techniques.	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 Melons Outcome 2 Strategy 2 and the KPI Development of pest and disease management strategies that mitigate crop loss in collaboration with growers	Sampling surveys in regions where SLM and ASLM are present testing monitoring protocols from MT16004. Sample collections looking at leafminers and parasitoid activity.	Previous monitoring protocol tool time consuming especially where leafminers highly present. Collections of leaf mines have revealed a wide array of parasitoids in different regions.
Improved management by identifying gaps in available chemistry, integration with current management practices and resistance issues		Large number of insecticide permits are available for leafminers in a wide range of crops. Insecticides ranked according to toxicity to beneficial parasitoids.	Growers feedback on what is working and what they would like. New chemistries being sought by agronomists to help growers. Resistance testing showing not all insecticides are effective as part of project AS20002
Increase in knowledge of SLM and how Integrated Pest Management can improve the management of SLM with minimum use of insecticides. A better understanding of the role of beneficial insects in IPM practices		Mini-literature review (Appendix 2.1) on monitoring for leafminers and the experiences overseas. Surveys have shown the range of parasitoids present in different regions	Grower and Industry M&E survey responses (Appendix 4.2) Workshops to show growers and agronomists what to do and look for and discussions on IPM of this pest and how to conserve the beneficial insects that attack them.
Increased awareness of the significance of this pest through host range information and assessment of crop impacts in affected regions.		Workshops, field days, farm walk and farm visits etc to raise the awareness of these pests to growers and agros. Provide handouts relevant to regions and crops.	Workshop survey forms, particularly between May and November 2023.
Build upon MT16004's outcomes of increased awareness of serpentine leafminer identification, surveillance and impacts, and extend to increased awareness of appropriate management within Australian systems.		Enhanced molecular identification tools qPCR and LAMP to distinguish between Liriomyza spp. Strengthening eDNA for empty leaf mines for all 3 leafminers. Awareness of IPM as viable approach to leafminer management with emphasis on parasitoid awareness through surveys.	Participation at workshops
Greater understanding of the spread and establishment potential of serpentine leafminer in Australia by collecting fine scale spread and seasonal pest activity data and producing pest forecasts.	**Vegetables** Outcome 1 Strategy 2 and the KPI - Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers **Potatoes** Outcome 2 Strategy 1 and the KPI Pest and disease management strategies are developed that mitigate crop loss in collaboration with	Surveys conducted in regions where SLM and ASLM are present and using a centralised location for data to be used for modelling purposes.	Leafminer online portal. Journal article on special distribution of VLM, SLM and ASLM as well as w commonly found parasitoids.
Increased capacity for rapid, in-field identification of serpentine leafminer through validation of in-field diagnostics and specificity for serpentine leafminer and use of this rapid diagnostic method by diagnostic laboratories.		LAMP developed for SLM and validated using qPCR. Primers designed for SLM identification comparing with local native Agromizidae leafminers.	Paper being reviewed for publication. Training undertaken for researchers from QDAF staff.
Improved surveillance and management through the development of robust		Sampling surveys in regions where SLM and ASLM are present testing monitoring	Previous monitoring protocol tool time consuming especially where leafminers

<table>
<tr><td>monitoring and sampling protocols to give greater confidence in management actions.</td><td rowspan="4">growers

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

Melons
Outcome 2 Strategy 2 and the KPI Development of pest and disease management strategies that mitigate crop loss in collaboration with growers</td><td>protocols from MT16004. Sample collections looking at leafminers and parasitoid activity.</td><td>highly present. Collections of leaf mines have revealed a wide array of parasitoids in different regions.</td></tr>
<tr><td>Improved management of serpentine leafminer by identifying gaps in available chemistry and how beneficial organisms fit into a broader management program.</td><td>Large number of insecticide permits available for leafminers in a wide range of crops. Insecticides ranked according to toxicity to beneficial parasitoids.</td><td></td></tr>
<tr><td>Increased awareness of serpentine leafminer by providing identification and surveillance guides and improving grower and industry knowledge about the potential impacts</td><td>Providing relevant handouts and hands on experience about leafminers at workshops etc</td><td>Positive feedback form workshop surveys.</td></tr>
<tr><td>It is anticipated that the improved knowledge of SLM, as it specifically relates to Australian horticultural industries (encompassing host range, monitoring and sampling protocols, management guidelines and rapid diagnostics) will play a role in minimising the potential economic impact of this pest on these cropping systems, either through yield and/or quality penalties or crop input requirements.</td><td>Growers and agronomists more aware of SLM and ASLM through workshops etc and the role that beneficial insects play in combatting these pests so that there is less reliance on insecticides to manage their insect pest issues.</td><td></td></tr>
</table>

Monitoring and evaluation

Table 5. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes? **End of project outcomes** (from project logic) 1. Increase in knowledge of SLM and ASLM and how Integrated Pest Management can improve the management of SLM with minimum use of insecticides.	There is clear evidence from the Cesar interview survey (appendix 2.3) and the Nth Australian workshop series group discussion responses to Q2 &3 (appendix 4.3) of an increase in knowledge on how to use IPM to improve the management of the exotic leaf miner flies. This was especially evident in regions that had received multiple engagements by the project. The discussion Q4, on the use of currently permitted chemicals in terms of impact on beneficial insects and hence management outcomes, demonstrated an increase in knowledge of possible consequential negative outcomes from the use of different chemical groups.	• Continuous engagement Locations with multiple engagements by MT20005 and MT16004 clearly showed that the level of understanding and implementation was dependant on repeat exposure to the key IPM messages of these projects. This engagement and support needs to continue so all regions develop robust and responsive IPM farming systems for current and future chemical resistant pest incursions by building on current knowledge and experience, regardless of the starting level of understanding. Most VegNET regional plans include IPM which provides a strong existing vehicle for this continued engagement.
2. A better understanding of the role of beneficial insects in IPM practices.	The improvement in understanding of the role of beneficials was evident from all participants. Agronomists	• Updating monitoring and further developing diagnostics tools

	demonstrated a sophisticated understanding of beneficial insects in farming systems and openly shared their thoughts on integrating the IPM needs for SLM and ASLM into current growing systems. (appendix 2.3 & 4.2) Recent experience from the participants with FAW IPM strategies reinforced this understanding of beneficials in farming systems. Growers understanding of these principles varied from nil to well experienced in implementing IPM. All growers engaged in Nth Australia reported a better understanding of the role of beneficial insects in IPM systems.	The monitoring and diagnostic tools developed in these projects need to be continuously updated. LAMP primers for all exotic leaf miners are required. • Review of permitted chemicals As more efficacy and chemical resistance data becomes available the current permits will need to be reviewed and those appropriate to the ongoing IPM strategies renewed.
Intermediate Outcomes 1. Increased awareness what effect insecticides have on SLM and ASLM and the parasitoids that use them as hosts.	Nth Australia Discussion Q4 demonstrated the increased awareness of the effect of insecticides on the leaf miner parasitoids. The Cesar interview survey in NSW highlighted the need for clear advice in the use of chemicals to manage the pests which shows that agronomists are aware of the ramifications of insecticide use on these pests.	• Continued chemical resistance and efficacy research The full suite of resistance to insecticides still needs to be determined especially against newer, softer, and more targeted chemical options. Efficacy data on more insecticide groups needs to be investigated to provide more options to commercial produce with minimal damage specifications.
2. Being able to identify the active SLM and ASLM populations by looking at leaf mines, using monitoring tools and rearing techniques.	The Qld, NT and WA workshops used local examples of leaf mines where possible, with preserved pest and parasitoid samples and used field and high-powered video microscopes to instruct growers and agronomists. This approach was very effective with the Vietnamese growers in Carnarvon.	• Survey different vegetable producing regions to determine what parasitoids are present attacking local native leafminer populations.
3. Improved management by identifying gaps in available chemistry, integration with current management practices and resistance issues	Nth Australia Discussion Q4 highlighted the range of expertise in implementing IPM strategies for leaf miners and other exotic pests such as FAW. There is anecdotal evidence from Survey Q3,4&5 that attendees at workshops considered that IPM approaches are working but they need to be underpinned by regionally specific information. The efficacy and application of newer chemicals was highlighted in Discussion Q4. Ready access to, and use of improved diagnostics Discussion Q2 was seen as desirable in most regions.	• Continued research into more chemical options that will play a positive role into IPM management strategies.
How relevant was the project to the needs of intended beneficiaries?	The project continues to gain in relevance with the continued spread of SLM through the southeast of Australia and of ASLM in North. Without guidance, growers when first encountering these pests, can easily increase the severity of the incursion by inappropriate management. Nth Australia Survey Q13 and Discussion Q1 almost unanimous support from growers and industry support for committing their levy money to ongoing	• Monitor continued spread Due to the continued spread of SLM and ASLM into other growing regions, it is necessary to increase monitoring of pest and parasitoids and industry engagement to ensure newly affected areas have access to regionally relevant information and management options. • Continuously updated IMP and extension materials Growers and agronomists were fully supportive of continued improvement in

	related projects and providing updated information products. There were strong requests for translation of materials to increase the relevance to the non-english speaking background (NESB) growing community.	information available for leaf miner management.
How well have intended beneficiaries been engaged in the project?	The communication and engagement strategy produced in MS102 provided for a comprehensive engagement program across all mainland Australia vegetable and melon growing regions and using a range of engagement tools. The project utilised Webinars during the end of Covid and then used 2x conferences, 2x field days, 3x field walks, 44x farm visits, 3x market visits, 7x agronomist meetings, and 16x formal workshops. The project recorded 351 direct attendees over these activities, not including the conferences and field days. 227 growers and agronomists attended the formal workshops. Regions with a history of engagements with the project and the previous project MT16004, demonstrated a greater knowledge of appropriate management and had adopted or displayed willingness to adopt improved management strategies. They recorded extensive knowledge of and use of previous extension materials Survey Q 6 – 9. The enthusiastic response to farm visits and workshops from NESB growers in Geraldton, Carnarvon and Innisfail indicated they want more engagement in this space, Survey Q15. When presented with the extension material from MT16004 and MT20005 they were eager to incorporate the new knowledge but also wanted resources in language, if possible. Survey Q6-10 and Discussion Q1.	• Continued Engagement Mature growing areas need the opportunity to discuss and question best practice management that evolves from continued research. NESB growers need support, possibly through VegNET Regional Officers to develop more sophisticated Biosecurity and IPM focus. Survey Q3,4 and B1,2, &3
To what extent were engagement processes appropriate to the target audience/s of the project?	The early part of the project was still impacted by Covid restrictions, so the project produced webinars. This attracted the key growers and agronomists impacted in Qld and NSW. The interviews conducted by Cesar following these virtual engagements showed a high level of knowledge uptake and provided the project team with relevant questions from industry to be addressed by the project. Attendance at engagement activities in Nth Australia was recorded and clearly demonstrated that appropriate activities were selected and planned for each activity. For example, 95 growers and industry support personnel attended the Carnarvon workshop held in a leading growers shed and supported by the Carnarvon Vietnamese Association and Carnarvon Growers Association. Farm visits were more suitable in some	• Continued Engagement Select and conduct appropriate engagement and IPM learning activities for leaf miners and other highly resistant insect incursions.

	locations and allowed in-depth engagement with individual growers. Nth Australia Q14 responses indicted almost all attendees at every event would attend subsequent activities.	
What efforts did the project make to improve efficiency?	There was a major variation in Oct 2021 following detection of ASLM in WA and NT to increase leafminer surveillance, parasitoid monitoring, and an expanded Nth Australia engagement program to include growers from Carnarvon WA to Bowen QLD. This allowed the project to build on the knowledge and techniques already being developed for SLM and used existing project networks and expertise.	• Continued engagement with NESB growers. Translation of documents into appropriate language and follow up engagement is necessary to build on preliminary gains in knowledge and attitude to an IPM approach.

Recommendations

Understanding the biology, ecology and distribution of endemic parasitoids in Australia for use in biological control:
Research is warranted on *Hemiptarsenus varicornis* and *Opius cinerariae* to assess their potential role in controlling *Liriomyza* pests in open-air production as well as their potential suitability for augmentative biological control. Due to the low populations of *Diglyphus isaea* found in our surveys, the potential of this parasitoid for augmentative release may only be suitable for use in protected cropping situations rather than open-air production of vegetable crops.

Molecular diagnostics:
With the successful development of LAMP and Colorimetric diagnostics for SLM, extending this to both ASLM and VLM would help growers, agronomists and researchers quickly identify what species they are dealing with and whether they need to employ a modified management strategy accordingly. This will help to determine new incursions of these leafminers to different regions within Australia.

Surveys to find true distribution of these leafminers:
As more and more plant material, seedlings nursery stock and produce is distributed around Australia, ASLM and SLM are going to be found in more vegetable growing regions. Continued surveys of potential regions both intra- and interstate needs to occur for both leafminer activity as well as parasitoid presence. Parasitoids surveillance will help growers and agronomist understand what is present within their regions and how to manage them for when ASLM, SLM or VLM arrives in their area. Surveying both grower properties, and nurseries (seedling and ornamental) will help determine the spread of these pests.

Investigate the insecticide resistance status of *Liriomyza* pests:
Understanding the insecticide resistance status of Australian populations of VLM and ASLM would be highly valuable and would compliment the work being carried out as part of AS20002 which is looking at insecticide resistance in SLM. This can be undertaken with both bioassays and DNA technology as is being done through AS20002. Given the present (restricted) location of VLM in Australia, this is challenging. Establishing a VLM colony in its current location is the best way of sourcing fresh material for testing. This could be accomplished with the aid of the local community in far north QLD.

Chemical baseline data and resistance, and development of resistance management strategies:
Understanding the insecticide resistance status of Australian populations of ASLM, SLM & VLM will be key to ongoing management. In addition to determining any pre-existing resistances of the SLM incursion population, there is a need to generate baseline chemical sensitivity data for key insecticides which will be needed to monitor for future resistance evolution in the field. In the absence of established genetic diagnostics, the most likely approach to determine resistance is to conduct laboratory phenotypic bioassays. Resistance status should also be used to inform the creation of resistance management strategies that rely upon MoA rotations and IPM practices.

Chemical usage and permits: ASLM, SLM & VLM permits are in place covering multiple MoAs on most affected crop groups. However, additional permits may be required to ensure appropriate control options are available to growers of affected crops. Linked to this, there is a need to understand what chemicals growers are presently using and how this can be nuanced to ensure appropriate management programs are being employed. A demonstration site could prove to be quite powerful in this regard.

Development of economic thresholds and validation of monitoring methods (such as sticky traps and pupal trays):
Thresholds for pupa counts within trays, and sticky traps within field crops and closed cropping have not been developed within Australia for exotic *Liriomyza* pests. These techniques should be investigated properly and thresholds developed for key crops in the short term. Existing work on parasitoid biology and population modelling could be extended to develop thresholds for chemical pest management (i.e. when parasitoid presence is insufficient for pest control and pesticide applications become warranted).

Ongoing awareness of IPM options:
As demonstrated through this project, ongoing awareness raising activities will be critical to ensure uptake of management practices against *Liriomyza* leafminers in Australia. Extension activities could include hands on workshops, field days and demonstration sites, delivery of webinars, and the development and distribution of industry materials. All of this should be an integral part of a communication and engagement strategy used in any future project. Any future investment could also link with other projects that need to engage with growers and stakeholders to deliver joint project outcomes.

Refereed scientific publications

Journal article

Maino, J.L., Pirtle, E.I., Baudrot, V., Ridland, P. and Umina, P.A. (2023) Forecasting the potential distribution of invasive leafminer pests, Liriomyza spp. (Diptera: Agromyzidae), and their natural enemies. Austral Entomology. pp. 1–13. https://doi.org/10.1111/aen.12632

Xiaocheng Zhu[1*], David Gopurenko[1], Joanne C. Holloway[1], John Duff[2], Mallik B. Malipatil[3] (2023/24). Two independent LAMP Assays for rapid identification of the serpentine leafminer, *Liriomyza huidobrensis* (Blanchard 1926) (Diptera: Agromyzidae) in Australia. (Under review)

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MAINO, J. L., PIRTLE, E. I., BAUDROT, V., RIDLAND, P. M. & UMINA, P. A. 2023. Forecasting the potential distribution of invasive leafminer pests, Liriomyza spp.(Diptera: Agromyzidae), and their natural enemies. *Austral Entomology,* 62**,** 118-130.

PIRTLE, E. I., VAN ROOYEN, A. R., MAINO, J., WEEKS, A. R. & UMINA, P. A. 2021. A molecular method for biomonitoring of an exotic plant-pest: Leafmining for environmental DNA. *Molecular Ecology,* 30**,** 4913-4925.

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Intellectual property

All IP pertaining to the eDNA, modelling and app outputs are co-owned by Cesar Australia and Hort Innovation.

Note that the eDNA work is not for publication at the time of reporting. Cesar Australia are still hoping to develop this into a scientific paper.

There are no IP issues pertaining to the LAMP and Colorimetric work conducted by DPI NSW.

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10 The Royal Society, formally of Ausveg

Appendices

Appendix 1.0 Methodology

Appendix 1.1 Develop an in-field diagnostic test for serpentine leafminer

Appendix 1.2 Cesar leafminer ID service

Appendix 1.3 Two independent LAMP assays for SLM

Appendix 2.1 Monitoring to support integrated pest management of *Liriomyza* spp. pests in Australia

Appendix 2.2 Monitoring for serpentine leafminer in Australia

Appendix 2.3 Testing and refining monitoring protocols

Appendix 2.4 Forecasting the potential distribution of invasive leafminer pests, *Liriomyza* spp. (Diptera: Agromyzidae), and their natural enemies https://doi.org/10.1111/aen.12632

Appendix 3.1 Grower Guide for Melons and Cucurbits

Appendix 4.1 Northern Australia Extension Activities Report

Appendix 4.2 Grower and Industry M&E Survey Responses

Appendix 4.3 Evaluation of Northern Australian Extension Program

Appendix 4.4 Monitoring and Evaluation Report

Appendix 5.1 Summary of the modelling/app development within the Serpentine Leafminer Project https://apps.qonfluens.com/platform/login

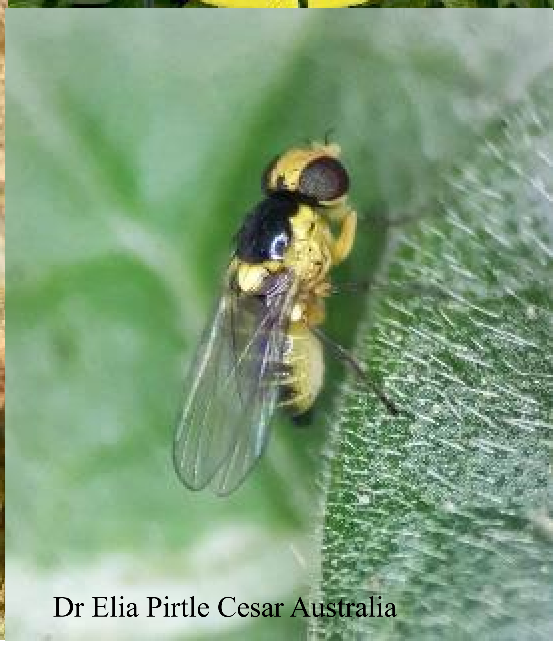
Appendix 6.1 Identification of parasitoid wasps reared from *L. huidobrensis* and *L. trifolii*

Appendix 7.1 Crop protection gap analysis, including how controlling Serpentine, American serpentine and Vegetable leafminers will fit into current management strategies and resistance

Appendix 1.0

Methodology

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*



Appendix 1.0

Methodology

This project built on the work of MT16004, critically adapting and developing targeted R&D specifically for SLM and subsequently ASLM in response to the incursions detected in late 2020 and mid 2021 respectively. The following methodology highlighted the proposed project activities and how the achieved outcomes have built on those from MT16004.

Component 1. Develop an in-field diagnostic test for serpentine leafminer
Led by Cesar (Dr Andrew Weeks).

Work already completed:
An existing qPCR assay for SLM (Sooda et al., 2017) was updated as part of MT16004, ensuring the assay would amplify SLM haplotypes from populations in Indonesia and Africa, but not other closely related exotic species (*L. bryoniae*, *L. trifolii*, *L. chinensis, L. katoi, L. sativae,* and *L. yasumatsui*) and closely related Australian species (*L. brassicae*, *L. chenopodii, Ophiomyia alysicarpi, O. solanicola, Phytomyza plantaginis, P. syngenesiae* and *Cerodontha* sp) (van Rooyan et al. in preparation). The qPCR assays (Real-time TaqMan® and PrimeTime™ assays) developed in MT16004 were further adapted into a novel environmental DNA (eDNA) approach for empty leaf mine samples (Pirtle et al., 2021b). Both the qPCR test for larvae and/or pupae and the leaf mine test can also be applied as an in-field diagnostic test. Results indicated that this test could be applied to larvae and/or pupae in the field. However, applying this test to leaf mines with no larvae/pupae resulted in mixed results due to inhibition of the qPCR.

Work Program:

1. *Improving outcomes for empty leaf mine samples:* The quick DNA extraction method for empty leaf mines was modified to address PCR inhibitors often found in plants, such as polysaccharides and phenolic compounds (Jobes et al. 1995), before the rapid in-field test can be applied reliably to leaf mines.

2. *Validating in-field test for SLM:* Samples of SLM were collected from multiple locations in NSW and QLD and preserved in 100% ethanol (sampling to be conducted by Qld DAF and NSW DPI as part of survey work). Leaf mines that contain active larvae as well as leaf mines that are empty were collected. Samples of larvae and leaf mines that are unlikely to be SLM were also collected from each location, by targeting host plants that are known to be of high preference to a native or naturalized leafminer, and are not known to be of high preference for SLM. Any native or naturalized agromyzid species collected were preserved in 100% ethanol to allow additional qPCR assay specificity testing against species not already tested during MT16004.

3. *Application - Confirming host plant and geographic range for SLM:* Samples of leaf mines from a wide variety of hosts, including those expected to be caused by SLM, and those which are uncertain, were collected across the range of SLM in Australia, to allow screening via the lab-based SLM qPCR assay (which was validated on empty leaf mines during the MT16004 project), allowing for the current host range of SLM to be better understood. This work now included the ASLM.

4. The NSW DPI DNA barcode facility (led by Dr. David Gopurenko) at Wagga Wagga Agricultural Institute can provide two levels of additional research to this proposal:

    A. Parallel development of rapid and low technology in-field molecular diagnostics of SLM using isothermal amplification methods such as RPA or LAMP in conjunction with low cost lateral flow strips (infield positive/negative testing within a 1-hour time frame). For direct visualization of the test result in-field without expensive Genie machine, NSW DPI evaluated and subsequently shifted from the lateral flow strip method to a more efficient and secure colorimetric method.
    B. Develop and validate the specificity of DNA sequence probes essential for molecular diagnostics of SLM and thereby avoid replication of the issue of false positive identifications which has hitherto affected commercially available molecular diagnostic kits for identification of SLM in Australia. This

was done by expanding the database of *Liriomyza* DNA barcodes from related endemic fauna to allow development of high specificity SLM probes used in downstream molecular diagnostics of the species.

ASLM specimens were also collected and sent to both Cesar Australia and NSW DPI for molecular diagnostics work using qPCR and possibly the low technology in-field molecular diagnostics of ASLM using isothermal amplification methods such as RPA or LAMP in conjunction with colorimetric assay.

Component 2. Develop the surveillance protocol for serpentine leafminer
Led by Cesar (Dr Elia Pirtle) and supported by other parties.

Work already completed:
Leafminer surveillance guidelines (with a focus on VLM) were created during project MT16004 via a 'mock-surveillance' data collection approach (Pirtle et al., 2021a). These guidelines were validated using VLM populations in urban areas within the Torres Strait Islands and have been compiled into a review manuscript which covered VLM, SLM and ASLM with a goal to guide early detection, delimiting and area freedom surveillance programs. These surveillance protocols, aimed at supporting biosecurity, focused on the detection of leaf mines. However, given that SLM is now impacting crops in Australia, surveillance protocols for the purpose of management (i.e. standardised population monitoring protocols) will be required.

An additional key part of monitoring SLM in Australia will be the use of forecasting tools that can improve monitoring efficiency by targeting efforts to highest risk seasons. SLM activity potential across seasons in Australia was estimated during project MT16004, through the application of a novel, spatially-explicit simulation framework, called the Establishment, Spread, Impact and Management (ESIM) framework, which will be covered in Component 5.

Work program:
1. *Develop practical and standardised monitoring protocols for SLM infected areas:* Effective monitoring protocols must be able to distinguish between old leaf mining damage (for which management intervention is unnecessary) and active populations of SLM (which may require management intervention). The accumulation of leaf mine damage, which persists long past the lifecycle of the pest, can create misleading indications of leafminer activity in a crop. Monitoring for population activity, rather than solely leaf mine presence, is a key component of IPM programs overseas to ensure unnecessary chemical applications are avoided (for instance, when mines are present, but pest populations are absent due to parasitism or weather changes). The use of in-field monitoring techniques developed overseas, such as yellow sticky traps, pupal trays, leaf mine counts and rearing vessels for the pest and parasitoids, were developed into standardised and practical protocols for monitoring SLM in Australia for growers and consultants, and were demonstrated at workshops in the various growing regions that have SLM and ASLM activity. Field demonstration sites exploring the interactions between chemical use patterns and parasitoid presence and abundance were initiated, one of which was washed away in flood waters and the other used as a host preference study.

2. *Validating the establishment model and seasonal risk forecasts:* Real-time seasonal risk forecasts will help growers better understand risk before impacts occur and prioritise pest and beneficial monitoring efforts accordingly. Previous SLM seasonal risk research conducted under MT16004 was based on historical climatic data and biological data on SLM available through the international literature. In this project we utilised real time weather data to forecast seasonal risk potential and validate estimates against available Australian seasonal activity data for SLM. This data collected as part of other project research activities. This helped capture spread within states; periods of peak activity; effect on different crops; presence of parasitoids; performance of chemicals; and very importantly other pests requiring treatment such as Fall Army Worm. DAF Qld and NSW DPI contributed to the validation of seasonal activity predictions by providing available data collected through ongoing routine surveillance and reports from grower networks, standardised due to the developed monitoring protocols and thus more suitable for analysis and interpretation.

3. *Creation of SLM online portal with updating seasonal pest forecasts*: Seasonal SLM pest forecasts are available to growers and industry through a user-friendly web interface. These visual outputs aim to increase grower understanding of pest seasonality and critical monitoring periods.

Component 3. Develop an industry management plan for serpentine leafminer, ensuring that synergies and conflicts with management for fall armyworm (Spodoptera frugiperda) are taken into consideration
Led by AUSVEG and supported by other parties.

Work already completed:
Several outputs created as part of MT16004 can be leveraged to develop an industry management plan for SLM, including regional activity prediction tools (requiring validation as part of Component 5), thorough reviews of chemical options (including a Bowen and Bundaberg area-wide-management case study), and biological control options, which were summarized into a short leafminer management guide and regionally specific preparedness guides.

- Leafminer management guide:

https://ausveg.com.au/app/uploads/2020/07/1303CR2_Management-guide_FINAL_150620.pdf

Work program:
AUSVEG have been developing leafminer industry management plan which when complete will include *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii*. This will be a live document in line with other previously developed documents for tomato potato psyllid (TPP) and Cucumber green mottle mosaic virus (CGMMV) and include many aspects of managing the pest by the industry, engaging with relevant stakeholders to ensure effective management of the pest and business and trade continuity. It will serve as a guide to each of the contributing industries as a whole on managing the impacts of *Liriomyza* leafminers on their industries. This is only partially complete and will be formatted into a usable document in the new year.

AUSVEG have also been developing four commodity grower guides to address the differences in management considerations that occur between different cropping systems. These commodities will include vegetables, potatoes, melons, and onions. Available overseas management learnings have be used to guide the appropriate scopes of each guide. The regional preparedness plans developed within MT16004 and the enterprise management plans developed under the transition to management phase for the TPP incursion response have provided a basis to develop these crop focused industry grower guides. Because different cropping systems would be able to withstand varying levels of infestation and damage before an economic threshold is reached and action is required to manage the pest, these grower guides will try and reflect these differences. The grower guide for “Melons and other cucurbits” is nearing completion and will be a template for the other 3 guides. These guides also include ASLM as well as VLM as all three *Liriomyza* species are currently in Australia.

Component 4 and 8. Management and engagement strategy and associated extension material (workshops face-to-face and/or webinar, factsheets, podcasts) to drive educational material to growers and regional biosecurity and extension agents (VegNET), and to demonstrate in-field diagnostics and surveillance protocols – linking with Hort Innovation Extension and Communications team and industry extension projects.
Led by AUSVEG and supported by other parties.

Work already completed:
A number of extension and communication resources were developed under Project MT16004, largely around raising awareness of exotic leafminers, including SLM. These resources can be found on the AUSVEG and Cesar Australia websites. They provide an excellent platform to efficiently and effectively extend relevant information to industry, and to incorporate new findings from this project.

Work program:
New extension materials focusing on developing four grower guides as indicated in Component 3. This work has included focused workshops utilising the range of available and existing communication networks within the industry.

16 workshops were co-ordinated by AUSVEG and Qld DAF, in liaison with VegNET officers both in infested regions as well as a high risk region such as Victoria, Carnarvon and Geraldton in WA. In already infested regions the focus was on supporting growers to manage serpentine leaf miner and American serpentine leafminer infestations including IPM regimes and in the high risk region, a focus on surveillance and preparedness. A key component of extension was working with local agronomists who can 'champion' IPM monitoring techniques.

The content of the workshop was determined by the team leader from Qld DAF, who conducted a workshop for agronomists in the Granite Belt in December of 2022. This became a blueprint of the workshops to be rolled out across the North. These workshops consisted of both information exchange, grower discussion and practical sessions looking at the various stages of the leafminer life cycle and the range of parasitoids that attack the leafminers and how to monitor for them. Information about chemical use and an evaluation of the project and content of the workshops was also sought.

AUSVEG leveraged the existing networks within the industry as part of the communication and extension program, in planning for these workshops. These networks included predominantly the VegNet RDO network for the vegetable industry, as well as regional farmer bodies and the agronomist networks. Existing communication channels were utilised on a needs basis and included AUSVEGs weekly newsletter, Vegetables Australia and the newsletters of AMA, NGIA, OA.

The creation of a demonstration site to show the effect of inappropriate chemical management (i.e such as the imagery found in Chirinos et al. 2017) would have been an extremely valuable extension exercise if it had not been washed away. The host preference study planted in the Spring of 2023 did attract interested growers and agronomists.

Extension activities were significantly expanded to including all three leafminer species (*L. huidobrensis*, *L. sativae*, and *L. trifolii*) into the industry management plans, including strategies to mitigate the spread of *L. trifolii* further south. This will ensure that they are aware of the pest, recognise symptoms, understand the avenues for reporting, and through education, limit further spread to support containment of the pest. With the incursion of ASLM, the extension effort was designed to slow the southward spread of the pest toward the major production regions, containing it in the northern part of the country. This was done by planning and executing grower and community engagement, by disseminating knowledge of the pest, its symptom recognition and management as a result of findings from the current project towards.

The workshops were undertaken across Western Australia, Queensland and the Northern Territory, along with additional one-on-one grower engagements. To ensure legacy of the developed research, the coordinator engaged with and helped train a range of stakeholders on the management of SLM, ASLM, VLM. These included VegNET officers, biosecurity extension officers and personnel, state DPI stakeholders, NAQS and rangers, agronomists, schools and community gardens.

Component 5. Develop a spread model for serpentine leafminer in Australia using fine-scale spread data, and incorporating this data into seasonal pest-risk forecasts
Led by Cesar (Dr James Maino).

Work already completed:
Spread and impact risk predictions for VLM, ASLM, and SLM in Australia were estimated during project MT16004 via the aforementioned Establishment, Spread, Impact and Management (ESIM) framework. The exploration of both human-assisted dispersal and natural short-ranged dispersal via the ESIM framework identified high risk incursion locations and susceptible production regions (Maino et al. in preparation b). This analysis was used to target extension work within project MT16004. However, due to poor availability of fine-scale spread data for exotic *Liriomyza* spp. available in published international studies we had to utilise spread data from the similarly small fly, *Drosophila suzukii*, which introduces a level of uncertainty to spread forecasts. Consultation with

government biosecurity offices also identified a future need for these models to be developed at finer resolutions so they could inform farm scale delimiting activities or management responses.

Work Program:

1. *Validating the spread model and extension to field management*: Previous SLM spread research under MT16004 focused on national scale spread patterns (i.e. 9 km resolution) to inform broad preparedness priorities. Under this investment we endeavoured to validate these models based on the observed rates of spread throughout the project. However, we also extended previous spread models to finer spatial resolutions to address field scale management priorities, such as parasitoid recolonisation rates and associated delays in pest suppression. Such analysis established the groundwork for simulations of the effectiveness of augmentative biological control through mass rearing and release of parasitoids. NSW DPI and DAF Qld contributed to the predictive spread model validation by providing surveillance data collected while documenting spread during the incursion response to SLM within NSW and QLD and through their survey work on farms.
2. *Creation of SLM online portal*: The SLM portal containing seasonal SLM pest forecasts (described in Component 2) includes pest/beneficial population spread outputs. These visual outputs aim to increase grower understanding of pest risks, as well as confidence in proper chemical management in the context of managing parasitoids and pest population and spread.

Component 6. Survey parasitoids of serpentine leafminer in affected regions and indicate how beneficial insects can be incorporated into a broader pest management plan

Led by QDAF and NSWDPI and supported by University of Melb (Dr Peter Ridland)

Work already completed:

The University of Melbourne and Cesar Australia hold a library of Australian parasitoid samples, representing several species attacking both non-pest leafminer flies and SLM's close relative, the vegetable leafminer fly collated through MT16004. Morphological identities have been provided by graduate students and overseas experts, and CO1 sequences have been obtained for each identified specimen, creating a valuable reference library for screening parasitoid samples collected from SLM in Australia. An extensive literature review was used to create a database of all parasitoid records currently known within Australia, as well as an occurrence map (within the VLM portal https://cesaraustralia.shinyapps.io/VLMportal/).

Work program:

1. *Surveys for parasitoids of SLM in Australia*: (Largely undertaken by QDAF and NSW DPI) Parasitoid collection surveys were conducted at multiple sites across NSW and QLD. Due to the relative short length of the project, some area coverage was undertaken, however there was regular revisits to a number of key sites in both states over the 2021/22 seasons – this helped enable us to capture seasonal variation in parasitism rates especially. For project efficiencies, these surveys also collected samples of larvae and leaf mines that were expected and not expected to be SLM and sent off for eDNA testing to help validate this diagnostic tool. Similarly, samples of larvae and leaf mines that are expected and not expected to be SLM were collected from each location, to investigate potential wasp reservoirs within native or naturalized leafminer populations.

2. *Identification of parasitoids*: The project team's morphological and molecular expertise helped to identify parasitoid wasps collected as part of this project. Wasp samples were sent to Peter Ridland for cataloguing and identification with some being sent overseas to experts in this field when specimens could not be identified locally. Not all samples were sent. Some were subsamples to allow for a collection to remain in both NSW and QLD to help with workshop training exercises.

3. Due to the subsequent introduction of ASLM in the north of the country, additional surveys were carried out in the NT and WA for ASLM. The survey work allowed the NT and WA partners to gather more information on the spread of this new exotic pest and the host preference including alternate hosts such as weeds species during the summer months when very little cropping was taking place.

Component 7. Crop protection gap analysis, including how controlling serpentine leafminer will fit into current management strategies and resistance. Working with the Hort Innovation Regulatory Affairs - Crop Protection Manager as appropriate
Led by QDAF and NSW DPI

Work already completed:
Detailed chemical and biological option reviews collated (including an area-wide-management case study of chemical use in Bowen and Bundaberg tomato crops) and chemical permits obtained with MT16004.

Key gaps remaining:
As this project progresses, a crop protection gap analysis was conducted to identify where additional R&D support was required. Some initial areas where gaps were identified included adequate coverage of affected crops with appropriate chemical permits; information necessary for area wide management approaches that account for all pests and chemical uses in a system to preserve beneficial insects; availability of economic thresholds and monitoring protocols for population size estimation.

Due to the extension to the project requested by Ausveg, there is a period of 8 months where no field or lab-based work would be undertaken on leafminers. This additional time allowed for some preliminary work on new and permitted insecticides, and was done in conjunction with AS20002: Management of insecticide resistance in serpentine leafminer (*Liriomyza huidobrensis*). This work was done at the Gatton Research Facility in the lab with potted plants and looked at imidacloprid and spirotetramat. This work will be fully reported on in AS20002.

Appendix 1.1

Develop an in-field diagnostic test for serpentine leafminer

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

Appendix 1.1

DNA extraction from insect and leaf mine samples

DNA was extracted from insect samples using the Qiagen DNeasy Blood & Tissue Kit (using the spin-column protocol). Total genomic DNA (gDNA) extractions were performed following the standard Qiagen DNeasy Blood & Tissue Kit protocol and using approximately an entire fly or larvae crushed with a sterile pipette tip. DNA was extracted from empty leaf mine samples using a modified Chelex extraction protocol (Walsh et al., 1991). Individual leaf mines up to 10mm x 2mm were excised and placed into 1.5 ml tubes along with a 3 mm glass bead (Retsch GmbH), 5 µl of proteinase K and 200 µl of 5% Chelex solution. Each tube was then shaken in a Tissue Lyser II (Qiagen) at 30 oscillations/s for 1 min. Samples were subsequently digested at 55°C for 60 min, followed by a final incubation at 95°C for 15 min with periodic vortexing. Extractions were stored at −20°C until required. Prior to real time polymerase chain reaction (qPCR) amplification, extractions were spun at 10,000 g for 2 min. Aliquots from the bottom half of the supernatant immediately above the Chelex resin was used for qPCR amplification.

For infield Biomeme tests DNA extractions were performed by crushing a single fly, larvae or 10mm x 2mm of excised leaf mine in 100ul Qiagen buffer AE with a sterile pipette tip. Four microlitres of this solution was transferred directly into the qPCR reaction.

eDNA assays development

Commented [JD1]: Can you also make a note that the eDNA work is not for publication. We're still hoping to put this into a scientific paper (time permitting).

Environmental DNA assays have previously been developed for *L. sativae, L. trifolii and L. huidobrensis* (Sooda et al. 2017) and *L. brassicae* (Pirtle et al. 2021). We initially tested these assays to determine their specificity and non-target leaf mine species. Initial in silico analyses of the *L. huidobrensis* assay suggested potential compatibility (matching primer and probe sequences) with Liriomyza *bryoniae*. Quantitative polymerase chain reaction (qPCR) subsequently confirmed non-target cross-amplification (see results). We therefore downloaded the complete mitochondrial genome sequences from Genbank (www.ncbi.nlm.nih.gov) for *L. huidobrensis,* L *bryoniae, L chinensis* all non-target leaf mine species to design species-specific assays. Unique regions were first identified by aligning all genomes in Geneious (vers. 10.2.5; https://www.geneious.com). Once target regions were identified, assays were designed using the custom TaqMan® Assay Design tool (https://www.thermofisher.com/order/custom-genomic-products/tools/cadt/) and primer and probe specificity was checked in silico using primerblast (https://www.ncbi.nlm.nih.gov /tools/ primer-blast/) with no non-target cross-amplifications identified. Species-specific TaqMan® copy number assays for each species were ordered from Thermo Fisher Scientific Inc. (Waltham, MA), labelled with FAM fluorophores. Once assays had been tested individually on all target and non-target gDNA samples (see below), we then transferred the assays to the PrimeTime? qPCR method (Integrated DNA Technologies) labelled with HEX and Cy5 fluorophores (Table 1) so that we could run all assays in a two multiplex reactions and detect all three fluorophores.

Additional validation of American serpentine leafminer assay

We screened samples as part of an *L. trifolii* empty leaf mine eDNA degradation experiment set up by Bhuwaneshwariba Vala (Northern Territory Government). Bhuwaneshwariba collected empty leaf mine samples and stored these over a time frame from 0 days to 28 days from bean plants in three replicates. A total of 18 samples were provided: +ve control, 0 days, 3 days, 7 days, 14 days and 28 days (3 replicates for each treatment). We were able to successfully detect *L. trifolii* eDNA in empty leaf mine samples out to 28 days. These results were in line with our earlier findings with *L. sativae* eDNA being detected in empty leaf mines out to 28 days (Pirtle et al., 2021).

qPCR assays - Roche LightCycler

Real-time TaqMan and PrimeTime qPCR assays were conducted using a Roche LightCycler 480 II system in a 384-well format. 10 mL reactions containing 5 mL of KAPA probe force PCR Master Mix (Merck), 0.5 mL of each TaqMan or PrimeTime qPCR Assay, 2.5 mL ddH2O, and 2 mL of template DNA were prepared in triplicate. Included in each 384-well assay plate were control reactions containing a tenfold dilution series from 1,000,000 to 1000 femtograms of gDNA and a negative control with no DNA template. The amplification occurred in conditions of 3 min at 98 °C, followed by 10 s at 95 °C and 20 s at 60 °C for 50 cycles. The amplification profiles of each PCR were used to determine the cycle quantification (Cq) value using the Absolute Quantification module of the LightCycler 480 II software package. A TaqMan Exogenous Internal Positive Control (VIC probe) was run for each sample to test for the presence of PCR inhibitors. No inhibition was detected on any eDNA sample. Quantitative PCRs were undertaken in a separate dedicated room, while environmental DNA extractions were undertaken in a room dedicated to low-quantity DNA (a separate room from where tissue DNA extractions were undertaken). Negative controls were included at all stages (DNA extraction, qPCR) so that contamination issues could be identified if present. No contamination was detected. A sample was considered positive if 1/3 qPCR replicates detected the target DNA.

qPCR assays – Biomeme three 9 system

Real-time TaqMan and PrimeTime qPCR assays were conducted using a Biomeme three 9 system in a 9-well format. 20 µL reactions containing 10 µL of KAPA probe force PCR Master Mix (Merck), 1 µL of each TaqMan or PrimeTime qPCR Assay, 5 µL ddH2O, and 4 µL of template DNA were prepared. Included in each run was a negative control with no DNA template. Positive control reactions containing a tenfold dilution series from 1,000,000 to 1000 femtograms of gDNA were run separately due to limitation of wells available. The amplification occurred in conditions of 3 min at 98 °C, followed by 10 s at 95 °C and 20 s at 60 °C for 50 cycles. The amplification profiles of each PCR were used to determine the cycle quantification (Cq) value using the Biomeme Go software package. A TaqMan Exogenous Internal Positive Control (VIC probe) was run for each sample to test for the presence of PCR inhibitors on the lighcycler as described above. Where inhibition was detected on any eDNA sample the sample was diluted 10 fold an re-run. Negative controls were included at all stages (DNA extraction, qPCR) so that contamination issues could be identified if present. No contamination was detected. A sample was considered positive if 1 qPCR detected the target DNA.

Primer efficiency, limit of detection (LOD) and quantification (LOQ)

Primer efficiency, limit of detection (LOD), and limit of quantification (LOQ) were assessed following the protocol and curve fitting method described in Klymus et al. (Klymus et al. 2020). For the standard curve, serial dilutions of gDNA derived from tissue extractions were prepared in elution buffer AE, Qiagen. The 10-fold dilution series spanned over five orders of magnitude, ranging from 100,000 to 1 femtograms of DNA, measured with a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Each dilution was run with ten replicates and reported Cq (cycle quantification) values were used to determine primer efficiency, LOD, and LOQ. Amplification efficiency was determined by plotting Cq values against gDNA dilutions and calculating the linear slope and the coefficient of determination (R2) value.

Specificity testing

We tested the specificity of each assay on 1 ng of gDNA extracted from insect samples from three individuals for each of 14 leaf mine fly species spanning 8 countries. The performance of each assay was tested on L. brassicae, L. bryoniae, L. trifioli, L. huidobrensis, Liriomyza chenopodii, Liriomyza chinensis, Liriomyza katoi, Liriomyza sativae, Liriomyza yasumatsui, Ophiomyia alysicarpi, Ophiomyia solanicola, Phytomyza Plantaginis, Phytomyza syngenesiae and Cerodontha milleri and Drosophila melanogaster. The qPCRs were performed as outlined above for each sample, with 3 negative controls, and was repeated when two of the assays were changed to PrimeTime assays with different fluorophores.

Field samples

We validated our field assay with 24 samples of L. brassicae collected from sites around Preston Victoria sampled leaf mines from Sow Thistle, Daisey and Narsturtium. We also used 16 samples L. Huidobrensis (8 larvae samples and 8 empty leafmine samples) collected from beans in Queensland.

Sequencing positive samples

Six field samples that returned a positive result for *L. brassicae* DNA were subsequently amplified by PCR using the short barcoding primers ArF5 and ArR5 (Gibson et al. 2014) tagged with M13 tail sequences. Products were then sequenced using Sanger sequencing (ABI 3730xl, Macrogen Korea) in dual directions using M13 primers and compared to reference sequences to confirm species haplotype authenticity and overall assay specificity.

Sequencing reference samples

All reference samples used as target and off-target controls were amplified by PCR using the barcoding primers LCO1490 and HCO2198 (Folmer et al. 1994) tagged with M13 tail sequences. Products were then sequenced using Sanger sequencing (ABI 3730xl, Macrogen Korea) in dual directions using M13 primers and compared to reference sequences to confirm species haplotype authenticity and overall assay specificity. For some samples where amplification with LCO1490 and HCO2198 was unsuccessful the short barcoding primers ArF5 and ArR5 (Gibson et al. 2014) or the leafminer specific primers LeafminerCOI-F and LeafminerCOI- R (Blacket et al. 2015) were used.

Table 1. Primers and labelled probe sequences targeting different regions of the mtDNA regions for *L. sativae, L. trifolii, L. huidobrensis,* L *bryoniae, L chinensis* and *L brassicae*.

Species	Common name	Gene region	Amplicon size (bp)	Primer/Probe sequence	Reference
L. sativae		CO1	109	Primer 1 5'- ACCCCCTGCTTTAACTCTTTT -3' Primer 2 5'- AGCACCACCATGTGCAATAA -3' Probe FAM- CAGTATAGTAGAAAATGGGGCTGGGA -NFQ	Sooda et al. 2017
L. trifolii		CO1	66	Primer 1 5'- CGGAGCTGGTACAGGATGA -3' Primer 2 5'- GAAGCTCCACCATGTGCAATA -3' Probe FAM- CCGTTTACCCTCCCCTTTCCTCA -NFQ	Sooda et al. 2017
L. huidobrensis		CO1	112	Primer 1 5'- CCTCCAGCTCTTACCCTTCTAC -3' Primer 2 5'- CTGAAGCTCCTCCATGAGCAA -3' Probe FAM- AAGAAGTATAGTTGAAAACGGAGCTGGGA -NFQ	Sooda et al. 2017
L. huidobrensis		ND5	132	Primer 1 5'- ATAAACTACCCATTCAGCTATCTAAT -3' Primer 2 5'- CATGACTTCCAGCAGCTAT -3' Probe FAM- CCCTGCCGTAACCAA -NFQ	This study
L. *bryoniae*		ND5	129	Primer 1 5'- AAAAACTCCCTAATTCTCTATCCAAT -3' Primer 2 5'- GGCTTCCTGCTGCAATAGC -3' Probe FAM- CCAGCTGTAACTAAAGTTG -NFQ	This study
L. chinensis		CO1	146	Primer 1 5'- CCCAGCACTTACTTTACTTTTATTAAGAAG -3' Primer 2 5'- TTCCTGCGAGATGTAAAGAGAA -3' Probe FAM- CTCCATGGGCGATTAC -NFQ	This study
L brassicae		CO1	63	Primer 1 5'- GCCGGAACAGGATGAACAGTTTAT -3' Primer 2 5'- AGATGCCCCACCGTGAG -3' Probe FAM- CCCCTCTCTTCTATTATTG -NFQ	Pirtle et al 2021

Table 2. Primer LOD/LOQ

Assay	R.squared	Slope	Intercept	LOD	LOQ	efficiency	efficiency %
L. sativae CO1	1.00	-3.38	41.01	5.56	22	1.976	98
L. trifolii CO1	1.00	-3.42	39.23	3.54	949	1.963	96
L. huidobrensis CO1	1.00	-3.41	40.00	75.80	82	1.965	96
L. huidobrensis ND5	1.00	-3.41	38.24	12.34	55	1.965	96
L. *bryoniae* ND5	1.00	-3.57	40.40	31.60	110	1.905	91
L. chinensis CO1	1.00	-3.54	38.34	3.60	7	1.917	92
L brassicae CO1	1.00	-3.41	40.60	3.54	17	1.964	96
L brassicae CO1* Biomeme three 9	0.98	-3.46				1.945	94

Application – confirming host plant and geographic range for SLM.

The qPCR multi-species assay was validated against a range of endemic and exotic leafminer species, including collections from different locations (Table 3). Species screened include; *L. brassicae, Liriomyza chenopodii, L. chinensis, L. huidobrensis, Liriomyza katoi, L. sativae, L. trifolii, L. bryoniae, Liriomyza yasumatsui, Calycomyza humeralis, Cerodontha milleri, Drosophila melanogaster, Ophiomyia alysicarpi, Ophiomyia solanicola, Phytomyza plantaginis, Phytomyza syngenesiae* and *Scaptomyza flava*. The assays showed good specificity for the provided specimens all samples that did not amplify for one of the six species specific tests were checked with sequencing. Using these assays, we screened 378 leaf miner flies, larvae, pupae, or empty mine samples collected from across Australia and globally. With CO1 sequencing of a subset of 148 of these being undertaken to confirm species identity and primer specificity.

Table 3. Specificity of multispecies qPCR assays confirmed on the following samples of target and non-target taxa.

Species	**Population**	**Number samples**
Calycomyza humeralis	Queensland	9
Cerodontha milleri	Victoria	4
Drosophila melanogaster	Fiji	3
Liriomyza brassicae	Victoria	5
	Indonesia	1
	Queensland	5
	Timor Leste	3
Liriomyza bryoniae	Europe	3
Liriomyza chenopodii	Victoria	4
Liriomyza chinensis	Indonesia	5
Liriomyza huidobrensis	Indonesia	5
	Kenya	4
	Queensland	125
Liriomyza katoi	Indonesia	1
Liriomyza sativae	Hawaii	3
	Indonesia	3
	Thursday Island	4

	Timor Leste	4
	Vietnam	3
	Kenya	1
Liriomyza trifolii	Fiji	3
	Indonesia	3
	Kenya	3
	Northern Territory	6
	Timor Leste	3
	USA	5
	Western Australia	95
	Solomon Islands	3
	Cape York, Queensland	11
Liriomyza yasumatsui	Indonesia	1
Ophiomyia alysicarpi	Queensland	4
Ophiomyia solanicola	Queensland	4
Phytomyza plantaginis	Victoria	3
Phytomyza syngenesiae	Queensland	12
	Victoria	3
Scaptomyza flava	Victoria	4
Agromyzidae sp.	Queensland	5

References

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Appendix 1.2

Cesar leafminer ID service

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

Image: Dr Frezzel Praise J Tadle Qld DAF

Image: John D Duff Qld DAF

Image: Dr Frezzel Praise J Tadle Qld DAF

Image: John D Duff Qld DAF

Cesar Australia

Identification of serpentine leafminer in Victoria

The once exotic serpentine leafminer (also known as pea leafminer) (*Liriomyza huidobrensis*) is now considered established in NSW, Queensland and, most recently Victoria, with the tiny fly detected on a vegetable farm in Werribee in winter 2022.

The serpentine leafminer is a major horticultural pest; the larvae tunnel through leaves, feeding and creating thick white trails, called 'leaf mines'. Most damage occurs at the larval stage.

Through a Hort-funded project, led by QDAF, Cesar Australia is offering a 'free' identification service for Victorian growers who suspect they may have serpentine leafminer on their farm. This service utilises genetic diagnostic capability allowing identification from the leaf mines that are evident on leaves.

Typical leaf mine - trails or 'mines' on the leaf surface.

Collecting & sending samples for identification

Collecting leaf mine samples

- Collect 5-10 individual mined leaves per crop. Aim for leaves that are fresh and heavily mined if possible.
- Place the leaves in a plastic container or Ziplock bag. A piece of tissue paper or paper towel can be placed into the container to absorb excess moisture.
- Leafminer species can be associated with certain host plants. It is therefore important to sample of leaf mines from each plant species in order to determine all species present.
- Adequate collection data including host plant and location are essential for successful testing. To assist us, please print the below form '***Cesar Australia leaf mine testing service – sample information***', fill out all the details and include this when posting your sample.

Sending samples

- Samples should be sent via overnight express post on Monday - Wednesday. Do not send samples towards the end of the week or over the weekend.

- Once samples have been posted, please notify us via email at avanrooyen@cesaraustralia.com This will ensure samples are processed in a timely manner.
- Samples should be addressed to:

Cesar Australia

Cesar Australia leaf mine testing service

Anthony van Rooyen
Cesar Australia
Level 1, 95 Albert St
Brunswick, VIC 3056

Acknowledgement: This service is supported through the Hort-Innovation funded project MT20005, using the vegetable, potato, melon and onion research and development levies and contributions from the Australian Government. This project is led by QDAF, with project partners including Cesar Australia, AUSVEG, The University of Melbourne, NSW DPI, NT DITT and WA DPIRD.

Cesar Australia leaf mine testing service – sample information

Collection date: Collector name:

Mobile: Email:

Grower Details

Grower name - ……………………………….

Paddock name - ……………………………….

GPS details / road address - ……………………………….

Paddock Details

Crop type - ……………………………….

Crop growth stage - …………………………………………..

Pesticides used - ………………………………………………..

Other notes

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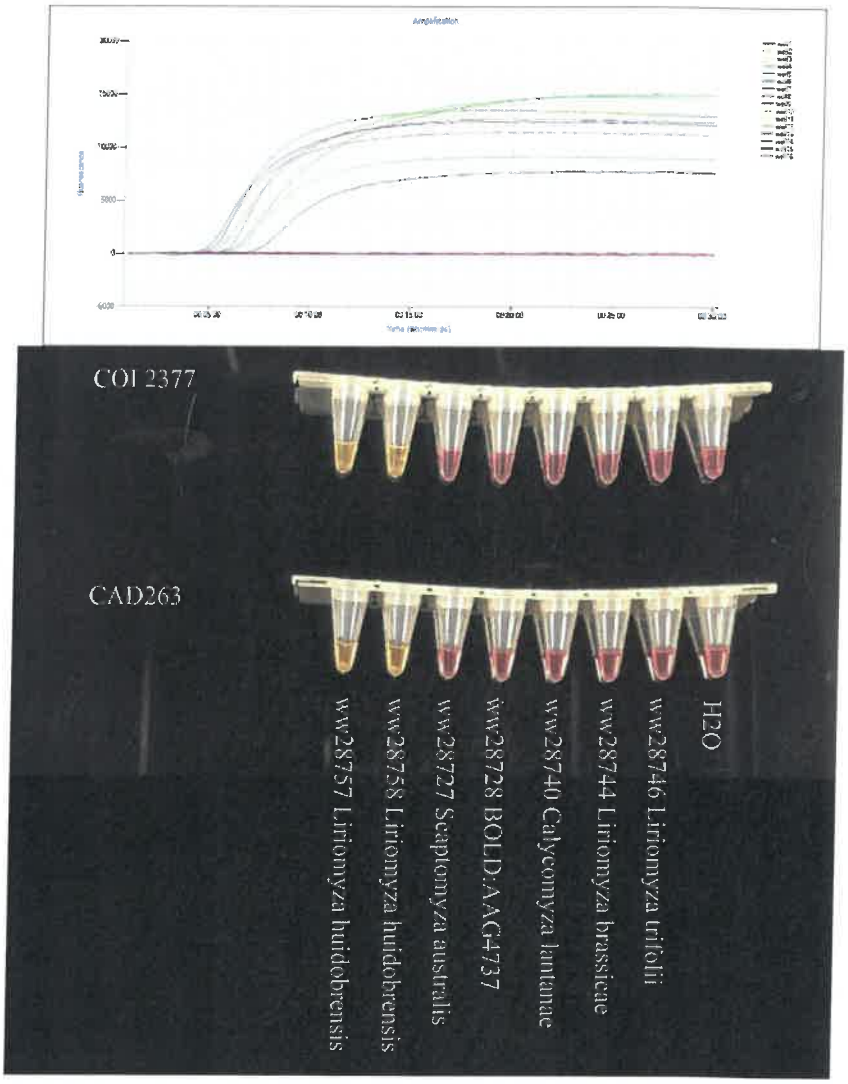
Appendix 1.3

Two independent LAMP assays for SLM

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*



Two independent LAMP Assays for rapid identification of the serpentine leafminer, *Liriomyza huidobrensis* (Blanchard 1926) (Diptera: Agromyzidae) in Australia

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Abstract: *Liriomyza huidobrensis* is a leafminer fly and significant horticultural pest. It is a quarantine listed species in many countries and is now present as an established pest in Australia. *Liriomyza huidobrensis* uses a broad range of host plants and has potential for spread into various horticultural systems and regions of Australia. Rapid in-field identification of the pest is critically needed to assist efforts to manage this pest. Morphological identification of the pest is effectively limited to specialist examinations of adult males. Generally, molecular methods such as qPCR and DNA barcoding for identification of *Liriomyza* species require costly laboratory-based hardware. Herein, we developed two independent and rapid LAMP assays targeted to independently inherited mitochondrial and nuclear genes. Both assays are highly sensitive and specific to *L. huidobrensis*. Positive signals can be detected within 10 min on laboratory and portable real-time amplification fluorometers. Further, we adapted these assays for use with colorimetric master mixes, to allow fluorometer free in-field diagnostics of *L. huidobrensis*. Our LAMP assays can be used for stand-alone testing of query specimens and are likely to be essential tools used for rapid identification and monitoring of *L. huidobrensis*.

Keywords: loop-mediated isothermal amplification, molecular diagnostic, in-field diagnostic, cytochrome *c* oxidase subunit I, carbamoyl-phosphate synthetase 2

Introduction:

Larvae of leafminer insects develop in, and feed on, parenchyma tissues between leaf surfaces of host plants, leaving behind distinctive mined tunnels and frass deposits. Leafminers can adversely affect host plant health by reducing leaf photosynthesis, increasing leaf decay, and allowing entry of diseases into hosts[1]. Leaf mining behaviours have evolved in four insect orders and are present in nine phytophagous fly families. They are prevalent among most of the around 3,163 species of Agromyzidae Fallén, 1823 that collectively feed off over 140 families of host plants[2-4]. Various leafminer Agromyzids are agricultural pests with some being highly polyphagous across economically important host plants and are therefore significant pests of quarantine importance to international trade. *Liriomyza huidobrensis* (Blanchard 1926), *L. sativae* Blanchard, 1938 and *L. trifolii* (Burgess, 1880) are prevalent among these significant pests.

These three leafminer species evolved in the Americas, but are naturalised pests in most other continents, including Australia where each has recently established in different regions[5-7]. They are collectively ranked as number 20 in the current Australian National Priority Plant Pests list[8]. Each pest is recognised as a significant risk to the production of a variety of economically important horticultural crops and ornamental plants. In particular, *L. huidobrensis*, commonly known as serpentine leafminer (referred herein as SLM), was identified during 2020 surveillance in the greater Sydney region of NSW as a novel invasive pest. SLM causes extensive foliar damage to commercial vegetable crops grown in the region including beans, cucumbers and Asian leafy greens[7].

SLM can affect a broad variety of agricultural, ornamental and weed host plants in Australia[7], many of which are also hosts used by the two other introduced *Liriomyza* pests in Australia[9]. The likelihood of spread of these *Liriomyza* pests into diverse agricultural and ecological systems and regions in Australia is high[9]. Subsequently affected agricultural and ornamental industries will need to develop tailored integrative pest management strategies to deal with each pest according to their biology and interactions with hosts and other leafminer species[10-13]. In this context, correct and rapid species identification of SLM under field conditions is critical for timely control and management of outbreaks, particularly if SLM disperses into new areas or onto novel host plants.

There are 18 naturally present *Liriomyza* species in Australia[14]. Endemic leafminer species are often not pest of agricultural concern. Cited occurrences of most of these species are scarce or only historically reported (refer Atlas of Living Australia; https://www.ala.org.au/). Direct identification of leafminer species in the field is difficult and subject to observer error. Readily observable leaf mines on host plants flags the presence of pest leafminer activity. In a few cases, the mine patterns and host identity may be indicative of a particular pest species[7,15,16]. In-field visual identification of adult Agromyzid leafminer species is not considered practical due to their small size (Agromyzids range in size from 0.9 mm – 5.6 mm) and the subtlety of morphological features used in their diagnostics. Many Agromyzid species lack a formal description, and most of the described species can only be distinguished from siblings by a few observable morphological characters. Furthermore, female adult and early instar Agromyzids generally lack species-specific features, and most species identifications are reliant on dissection of male adults and microscopic examination of their genitalia. Typically, during pest leafminer surveillance, species identifications require laboratory based taxonomic examinations of male adult flies either captured directly on hosts or raised from instars in leaf mines sampled from affected host plants. Development from egg to assayable adult in these latter instances can take 15-30 days[17,18], and this can delay an alert to the presence of a priority pest and subsequent management responses.

Alternatively, molecular genetic methods can provide species level identifications of leafminer flies and key Agromyzid pests. The maternally inherited mitochondrial cytochrome *c* oxidase subunit I (COI) gene has featured prominently as a targeted locus for molecular identification of some economically important Agromyzid species[19-21]. Nucleotide sequences of the 5' COI DNA barcode region[22] linked to vouchered specimens are reported for genetic identification of important leafminer pest species[19,23] . These sequence references have formed the basis for further development of laboratory and or point of need genetic diagnostic methods to identify invasive *Liriomyza* pests in Australia[24,25] and elsewhere[20,26,27]. Sequences of nuclear encoded genes, including 28S and carbamoyl-phosphate synthetase 2 (CAD), reported for phylogenetic analyses of some Agromyzids[3] and *Liriomyza*[28] offer additional advantages for species identifications of pest leafminers. Comparative sequence analysis of independently inherited mitochondrial and nuclear loci were used to identify morphologically cryptic *Liriomyza* species[28], and to test the direction of interspecific hybridisation between closely related *Liriomyza* species[29]. Recently, a quantitative Polymerase Chain Reaction (qPCR)-based molecular identification method was developed for *L. huidobrensis*[27], addressing a critical need for *Liriomyza* biosecurity. However, this qPCR method has only been tested on a limited number of non-targeted species and still requires validation to confirm its applicability in Australia.

Genetic methods used for pest species identifications, such as qPCR and nucleotide sequencing, can take hours to days of laboratory processing time. This delay, coupled with delivery and registration of specimens at laboratories, increases the time required to provide an accurate identification and substantiated alert to the presence of a pest. Rapid in-field genetic diagnostics is preferable for a quick test confirmation of suspected SLM intercepts, but currently such systems are at primary stages of development or require substantive and or costly hardware.

Loop-mediated Isothermal Amplification (LAMP) is a low-cost technique for confirmation and or detection of target organisms[30]. LAMP incorporates a suite of oligo-primers specifically matched to the DNA of a target organism, and is designed to rapidly amplify linked copies of the target DNA. LAMP is well suited for in-field species identification of targeted pest insects, as it can test crude DNA lysates run on low-cost equipment for simple visual signalling of positive and negative test results[31].

Here we report novel development and validation of sensitive LAMP assays for rapid and specific identification of SLM, against a selection of leafminer species sampled during recent surveys for SLM in Australia. Also, we report modifications of the assays to allow simplified in-field colorimetric visualisation of SLM LAMP test results.

Results

Assay design and optimization

We designed novel LAMP primers (Table 1) for the mitochondrial COI region and nuclear CAD region for *L. huidobrensis*. Both regions are highly variable among species (Figs. 1 and 2), with sufficient resolution to distinguish SLM from all sequenced species. The COI LAMP primer set (COI2377) targeted a 208bp region downstream from the standard 5' DNA barcode region, the CAD primer set (CAD263) targeted a 194bp region. Some primers in sets were modified with Locked Nucleic Acids (Table 1) to increase the melting temperature.

For both assays, primer ratios (F3/ B3: FIP/ BIP: Floop/ Bloop) were optimized at 1:6:3, with the final concentrations of 0.4, 2.4 and 1.2 µM, respectively. LAMP assays run using two different commercial isothermal master mixes (DR001 & DR004) were similar in duration to peak product amplification. However, the fluorescent intensity of assays was much higher when using master mix DR001. Subsequently, we used DR001 in all downstream LAMP assays.

Assay sensitivities

Sensitivities of the COI2377 and CAD263 LAMP assays were determined using synthetic gBlock DNAs. The COI2377 assay was able to detect a minimum of 1000 copies/µL of DNA, with an anneal derivative of 82.6 ± 0.07 ˚C (Figs. 3a and 4). The CAD263 assay was slightly more sensitive with the detection limit at 100 copies/µL of DNA (Fig. 3b). The anneal derivative of CAD263 gBlock DNA was 84.1 ± 0.14 ˚C (Fig. 4). Anneal derivatives of the gBlocks were 1-2 ˚C higher than that observed among SLM positive samples (Fig. 4). These gBlocks were used as positive controls with the concentration of 1X 10^6 copies/µL.

Performance of the LAMP assays

Both COI2377 and CAD263 LAMP assays positively amplified all 184 SLM specimens within 25 mins. Normally, positive signals can be detected within 10 mins. When run on Genie III, the anneal derivative of COI2377 on SLM is 81.1 ± 0.13 ˚C, while CAD263 assays had an anneal derivative of 83.0 ± 0.01 ˚C. These assays, when conducted on a qPCR machine such as MIC, had a 1-2 ˚C higher melting temperature compared to the annealing temperature on GenieIII in both gBlock and samples. Generally, the positive signals were detected within 15 cycles (6.25 mins) and 20 cycles (8.33 mins) for COI2377 and CAD263 assays, respectively. Our LAMP assays were both highly specific to SLM with no amplification from any of the 146 specimens of the 15 non-target species (Table 2 and Supplementary Table S1).

Multiplexes of the two LAMP assays initially tested on a single SLM specimen, consistently exhibited two distinct and equal intensity anneal peaks (Fig. 5a) when COI2377 and CAD263 primer ratios were set at 1:3 or 1:4 respectively. Multiplexes incorporating these primer ratios were inconsistently scored across 14 additional tested SLM specimens. In most replicates, the anneal peak of either of the two LAMP targets dominated the peak of the other (Fig 5b), with no apparent trend to this biased amplification between the targets and no confident means to score if both targets had positively amplified.

Naked eye monitoring with colorimetric indicator

Crude isothermal heating of test reactions and use of a colorimetric LAMP MasterMix for equipment-free visualisation of LAMP positive products was successfully achieved after 30 minutes at single-plex COI2377 and CAD263 LAMP assays. Yellow stained positive LAMP reactions observed among all tested SLM specimens (N=8) were readily discernible from the default pink stains observed in LAMP tests of negative controls and 12 non-target species (Fig. 6). Sensitivity of the colorimetric Master-Mix LAMP assays to gBlock targets was the same as that initially reported using the Optigene Isothermal Master mix (100 &1000 copies / µL at COI2377 and CAD263 respectively).

Discussion

We developed two LAMP assays (COI2377 and CAD263) for specific genetic identification of SLM, under laboratory and in-field conditions. We tailored oligonucleotide primers in our LAMP assays to target short fragments of mitochondrial COI and nuclear CAD genes, each containing an array of fixed nucleotide positions that are unique to the species. Our *in-silico* comparisons of the primer suites against reported sequences at public repositories (BOLD and GenBank) indicated the primers were 100% compatible with SLM accessions of the target gene regions, and collectively unmatched to accessions of other reported Agromyzids and leafminers. We directly tested the specificity of our LAMP assays against 184 adult SLM sampled from affected sites in NSW, NT, Qld and WA, and against 146 co-occurring specimens of 15 non-targeted leafminer species, including four other *Liriomyza* species. Positive LAMP detections were obtained exclusively from all tested SLM, and absent for all other tested taxa. We acknowledge that direct specificity testing of our LAMP primer sets was taxonomically limited mainly to species obtained recently at horticultural sites suspected to contain introduced leafminers. Subsequently our sample tested just a small portion of all possible leafminer species present in Australia. Further, many of the untested leafminer species in Australia also are unreported at public sequence repositories for the gene regions targeted by our LAMP assays. Consequently, our in-silico analyses also were taxonomically limited by availability of comparable taxa. This is a common issue affecting LAMP developments and validations. For target genera, such as *Liriomyza*, often sequence and specimen replicates are readily available for the focal pest species, but limited for other described taxa that are either rarely encountered or of restricted geographic distribution. Despite these shortcomings, our LAMP assays failed to amplify from other recently introduced *Liriomyza* pests in Australia (*L. sativae* and *L.trifollii*), common native *Liriomyza* (eg., *L. brassicae* and *L. chenopodii*) and other common leafminer taxa (eg., *Chromatomyia syngenesiae* and *Phytomyza plantaginis*). Both LAMP assays showed high SLM species specificity and sensitivity, and both can be used independently for rapid identification in Australia.

We designed two synthetic gBlock DNA fragments to accompany our LAMP assays. These gBlocks should be used as known quantity positive controls and are especially useful when annealing or melt curve analyses are performed following LAMP amplification. Compared to SLM LAMP products, amplicons of these gBlocks have a higher melting temperatures. Subsequently, suspected cross-contamination of test samples by gBlock positives can be readily detected. In addition, because gBlocks are synthesized short fragment DNAs, they are more stable than extracted specimen DNA. Therefore it is more reliable as a test templates for detection of false negatives resulting from degradation of LAMP primer/master mix stocks. We recommend using gBlock at a concentration of 10^6 or higher, to match the fluorescent intensity generally observed from fresh SLM specimens.

Our LAMP assays are reliable for diagnostic detection of SLM under laboratory conditions using standard fluorometric thermal cyclers, and also potentially under in-field conditions using crude heating equipment for isothermal heating and colorimetric staining for simple visual observation of test results. In contrast to that reported by Zhang, et al. [32], our colorimetric method did not reduce the sensitivity of our LAMP assays. However, it should be noted that this method does not allow annealing or melt curve analyses, which means contamination from the gBlocks or potential non-specific amplification could not be distinguished from a true positive.

As the COI2377 and CAD263 LAMP assays have different observable anneal derivative temperatures, we attempted to multiplex them as a simultaneous real-time PCR assay for use with q-PCR equipment. However, in most cases, either one of the assays dominated the reaction and the other only appeared as a shoulder peak (Fig. 5 b). This may be due to the available ratios of mitochondrial and nuclear DNA extracted from among individuals, or to other efficiencies inherent in the amplification of the targeted gene fragments. Regardless, this is not of high concern as both assays can be used independently for simplified genetic identification of SLM.

In conclusion, we have developed two genetically independent LAMP assays that are specific and reliable for rapid genetic identification of SLM. Both assays were validated with adult and pupal specimens, and we expect the assays will work equally well on the larval specimens. Paired with fast and simple DNA extraction protocols (i.e., Xtract) these assays can be performed in-field within an hour and without need of expensive equipment. This will significantly accelerate the ready use of this diagnostic tool where there is need for rapid confirmation of a suspected presence of the pest. Both assays exhibited similar sensitivity and amplification time, offering users the flexibility to choose either one for diagnosing suspected SLM. In addition, the combination of the COI2377 and CAD263 assays could be used to investigate potential interspecific hybridization of *L. huidobrensis* and other species. Early in-field diagnostics facilitated by our LAMP assays will allow faster management responses to incursions and movement of this pest species.

Methods

Sampling

Ethanol (> 90%) preserved adult or pupal leafminer fly specimens were provided to us by various agencies (see acknowledgements) involved with SLM surveillances in NSW, NT, QLD and WA during 2020-2023. The specimens were either captured as adults or raised as adults emerging from larvae/pupae sampled from leaf-mined host plants.

Following all non-destructive DNA analyses, morphologically identified specimens were accessioned for curation at the Biosecurity Collections unit at Orange Agricultural Institute (NSW Dept. of Primary Industries). Retrospective DNA barcode identification of all specimens was conducted at the Wagga Wagga Agricultural Institute using protocols reported in Supplementary Methods S1. We deposited details of specimen sample records (Supplementary Table S1), their DNA barcodes and other associated sequences as a dataset (DS-SLMWW) "SLM and leafminers Australia", released at the Barcode of Life Data (BOLD) systems repository (http://www.boldsystems.org/).

LAMP designs and laboratory preparations

We targeted the mitochondrial cytochrome *c* oxidase subunit I (COI) gene as a primary DNA barcode locus for LAMP assay development based on its reported utility for molecular identification of Agromyzid species[33]. We used the extensive library of Agromyzid COI sequence accessions at BOLD and GenBank in that development. Additionally, this provided us with a means to test species identities of our sequenced specimens against taxonomically associated sequence accessions reported at the two repositories.

In addition to COI sequences, we obtained CAD gene sequences (partial) of 31 Agromyzids reported at GenBank and used these as an additional targeted gene sequence alignment for Agromyzid species identification and SLM LAMP development. This single-copy nuclear encoded gene has been reported for genetic identification of species in *Liriomyza*[28] and other important Dipteran genera (eg., *Culicoides*)[34]. For this purpose it serves as an independent and bi-parentally inherited locus for comparative species analysis against the strictly maternally inherited mtDNA COI locus.

For the design of SLM-specific LAMP primers, we examined COI and CAD alignments to identify sequence strings containing variable nucleotide sites among Agromyzid species and conserved sites among SLM specimens. We used our in-house sequence library of Agromyzid species and sequences obtained from GenBank and BOLD for alignment, and PrimerExplorer version 5 http://primerexplorer.jp/e/index.html to design candidate LAMP primer-sets specific to the COI and CAD sequence of SLM using the default setting. All primers were synthesised by Sigma Aldrich (Merck, USA) with HPLC purification.

LAMP assay optimization

We prepared a primer master mix for each LAMP assay. The outer primers (F3 and B3), inner primers (FIP and BIP) and the loop primers (LF and LB) were mixed as per the following ratios 1:6:3, 1:8:4, 1:10:5 and 1:12:6. Each LAMP assay (total volume 25 µL) consisted of 14 µL of Isothermal Master Mix (DR001, OptiGene, UK), 10 µL of primer master mix at various concentrations and 1 µL of test template. We optimized the LAMP assays on a Genie III (OptiGene, UK) at a temperature of 65 ˚C for 30 mins followed by an annealing curve analysis from 98 ˚C to 73 ˚C ramped at 0.05 ˚C/s. After the optimization of primer concentration, we compared two Isothermal Master Mixes DR001 and DR004 (OptiGene, UK) both of which incorporate a fluorescent dsDNA intercalating dye. We selected the optimum conditions based on time of amplification and fluorescent intensity.

We evaluated the sensitivity of the LAMP assays using two gBlock DNAs (Table 1, IDT, USA) in 10-fold serial dilution from 10^8 copies/µL to 10 copies/µL. The sensitivity test was performed on Genie III with the optimized primer concentration and the assay condition as mentioned above. We recreated graphs of all amplification and derivative curves in this study using the data output from Genie III machine.

LAMP primer specificity

We tested the specificity of both LAMP assays against 330 specimens comprising 16 species (Table 2 and Supplementary Table S1), including 184 SLM. The taxonomic identification of most specimens (216 out of 330) was confirmed through their COI sequences. Of the remaining specimens 106 were morphologically identified *L. huidobrensis* and 8 from laboratory colony of *L. trifolii*. The LAMP assays were conducted on a Genie III using the optimized condition or on a MIC qPCR machine (Bio molecular system, Australia) for higher throughput. On MIC, the cycling condition was: 60 cycles of a single step cycle at 65 ˚C for 25s, followed by melt curve analysis from 73 to 98 ˚C with ramping at 0.05 ˚C/s. The amplification time was calculated as 25s × Cq value.

LAMP multiplexing

We used the above-mentioned 25 µL reaction system with 10 µL of primer mix consisting of COI and CAD LAMP primers in different ratios which ranged from 1:9 to 1:1. Initially, we multiplexed LAMP with a single SLM specimen on a Genie III to determine an optimal primer master ratio of COI and CAD. The optimal ratio was tested against 14 additional specimens of SLM, on a MIC qPCR.

LAMP colorimetric detection

We conducted COI and CAD LAMP assays using crude heating in a 65 ˚C water bath and colorimetric staining to simulate in-field LAMP testing without specialised equipment used for isothermal heating and post-run scoring. LAMP assays contained 12.5 µL of WarmStart® Colorimetric LAMP 2X Master Mix (New England Biolabs, Australia), 1.6 µM of the FIP and BIP primers, 0.2 µM of the F3 and B3 primers, 0.4 µM of the Loop primers, 1 µL of target DNA, diluted to 25µL total volume. LAMP reactions were run in 200 µL sealed microtubes and floated in a 65 ˚C water bath for 30 mins. Post-run reactions were visually scored for colours observed among known SLM positives, negative controls and non-target species. Sensitivity of these colorimetric tests to target SLM DNA was determined against gBlocks run for 30 minutes on a PCR machine (Eppendorf Mastercycler, Germany).

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Author contributions

X.Z., D.G., J.C.H. and J.D. designed the project. D.G., J.C.H. and J.D. obtained the research funding. The LAMP primer and gBlock design, laboratory optimisation and testing were conducted by X.Z. Leafminer fly samples were provided by J.D. and J.C.H. Critical specimens were morphologically identified by M.M. The manuscript was drafted by X.Z. and all authors critical reviewed and approved the final manuscript.

Competing interest

The authors declare no competing interests

Data availability statement

The sequence data and specimen details are available from GeneBank under accession number OR038431 - OR038697 and Barcode of Life Data systems as a dataset (DS-SLMWW).

Additional Information

Supplementary Information The online version contains supplementary material available at

Correspondence and requests for materials should be addressed to X.Z.

Figure legends.

Table 1. Details of LAMP primers and gBlock synthetic gene fragments designed in our study. F2 and B2 primer regions of FIP and BIP are bold. Locked Nucleic Acids were marked with [+]. Lower case bases in the gBlock sequences are our modifications to increase the annealing temperature.

Table 2. Leafminer species tested for both COI2377 and CAD263 LAMP assays. Refer to Supplementary Table S1 for detailed specimen information.

Fig. 1 COI2377 LAMP primer anneal sites in partial COI alignment of *Liryomyza huidobrensis* and six other species of leafminer. Reverse primer sites are underlined. Arrows indicate the extension directions. Dotted line separate adjunct primer sites.

Fig. 2 CAD263 LAMP primer anneal sites in partial CAD alignment of *Liryomyza huidobrensis* and ten other species of leafminer. Reverse primer sites are underlined. Arrows indicate the extension directions. Dotted lines separate adjunct primer sites.

Fig. 3 Detection limits of COI2377 (a) and CAD263 (b) LAMP assays evaluated using gBlock synthetic gene fragments with serial dilutions from 1×10^8 copies/µL to 1×10^1 copies/µL.

Fig. 4 Comparison of the anneal derivations of gBlock positive control and *Liryomyza huidobrensis* DNA (sample ww28757) in the COI2377 and CAD263 LAMP assays. The COI2377 and CAD263 gBlock positives annealed at 82.69 and 84.40 °C, respectively. By contrast, for *L. huidobrensis* (sample ww28757), COI2377 and CAD263 LAMP products annealed at 80.98 and 83.00 °C, respectively.

Fig. 5 Anneal derivatives of multiplexed COI2377 and CAD263 LAMP assays. a) single *Liryomyza huidobrensis* specimen assay exhibiting separate and similar intensity derivative peaks for COI2377 and CAD263 targets (primer master mix target ratios of 1:4 and 1:3; run on Genie III). b) multiple *L. huidobrensis* specimen assays exhibiting separate but variable intensity derivative peaks for COI2377 and CAD263 targets (primer master mix ratio 1:3; run on MIC).

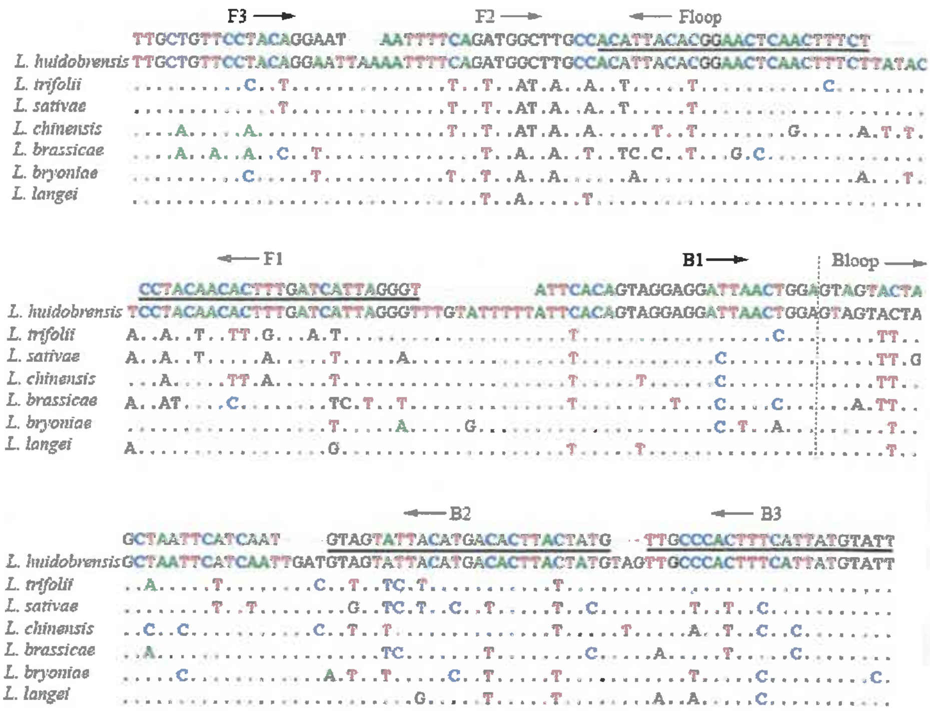
Fig. 6 Sensitivity (a) and specificity (b) of the CAD263 and COI2377 LAMP assays using colorimetric mastermix. a) Sensitivity tests of the CAD263 (top) and COI2377 (below) LAMP assays on a 10× dilution series from 10^8 to 10^1 copies/µL. b) Specificity tests of the CAD263 (top) and COI2377 (below) LAMP assays. Samples tested from left to right were ww28757 *Liryomyza huidobrensis*, ww28758 *L. huidobrensis*, ww28727 *Scaptomyza australis*, ww28728 *Tropicomyia polyphyta*, ww28740 *Calycomyza lantanae*, ww28744 *L. brassicae*, ww28746 *L. trifolii* and no-template negative control. End-of-run positive reactions exhibited as a yellow colour change, negative reactions unchanged pink colour. Refer Supplementary Table S1 for detailed specimen information.

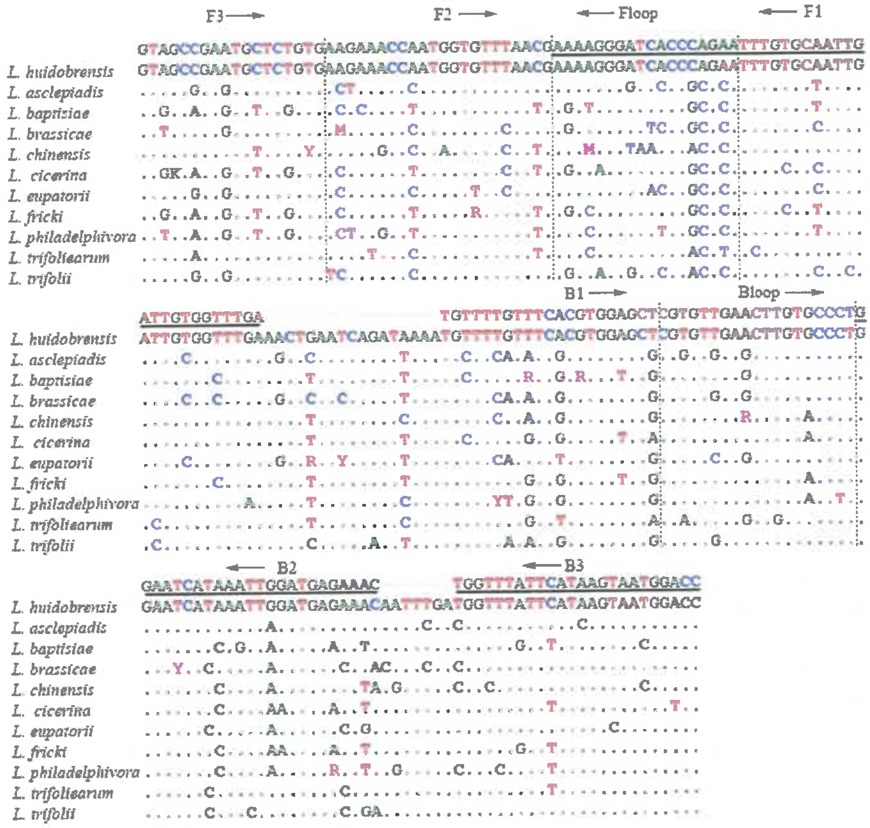
Table 1. Details of LAMP primers and gBlock synthetic gene fragments designed in our study. F2 and B2 primer regions of FIP and BIP are bold. Locked Nucleic Acids were marked with [+]. Lower case bases in the gBlock sequences are our modifications to increase the annealing temperature.

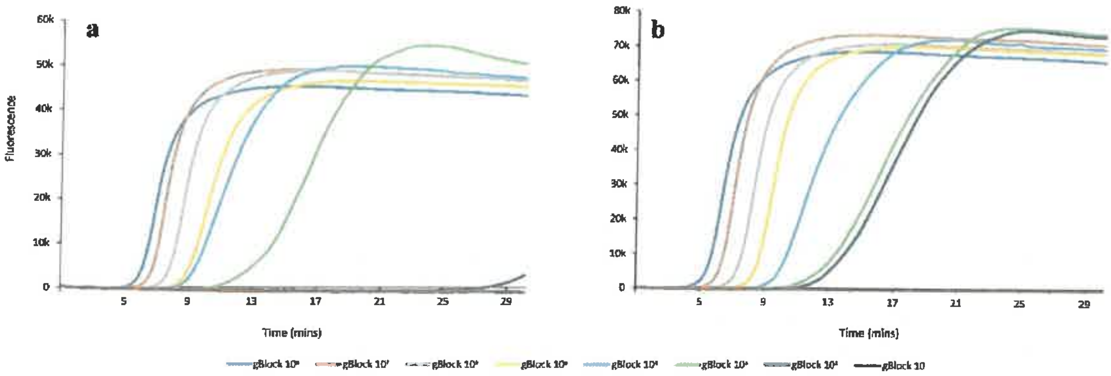
Primer name	Sequence (5'-3')	Gene	Target sequence
SLM_COI2377_F3	TTGCTGTTCCTAC[+A]GG[+A]AT	COI	208bp
SLM_COI2377_B3	AATACATAATGAAAGTGGGCAA	COI	
SLM_COI2377_FIP	ACCCTAATGATCAAAGTGTTGTAGGA **ATTTTCAGATGGCTTGCC**	COI	
SLM_COI2377_BIP	ATTCACAGTAGGAGGATTAACTGGAC **ATAGTAAGTGTCATGTAATACT[+A]C**	COI	
SLM_COI2377_LF	[+A]GAAAGTTGAGTTCCGTGTAATGT	COI	
SLM_COI2377_LB	GTAGT[+A][+C]TAGCTAATTCATCAAT	COI	
COI gBlock	TTGCTGTTCCTACAGGAATggggTAAAATTTTCAGATGGCTTGCCggggACATTACACGGAACTCAACTTTCTTggggATACTCCTACAACACTTTGATCATTAGGGTTggggTGTATTTTTATTCACAGTAGGAGGATTAACTGGAgggGTAGTACTAGCTAATTCATCAATggggTGATGTAGTATTACATGACACTTACTATGgggTAGTTGCCCACTTTCATTATGTATT		
SLM_CAD263_F3	GTAGCCGAATGCTCTGTG	CAD	194bp
SLM_CAD263_B3	GGTCCATTACTTATGAATAAACCA	CAD	
SLM_CAD263_FIP	TCAAACCACAATCAATTGCACAAAAA **GAAACCAATGGTGTTTAACG**	CAD	
SLM_CAD263_BIP	TGTTTTGTTTCACGTGGAGCT**GTTTCTCATCCAATTTATGATTCC**	CAD	
SLM_CAD263_LF	T[+T]C[+T][+G]GG[+T]GATCCCTTTT	CAD	
SLM_CAD263_LB	CGTGTTGAACTTGTGCCCT	CAD	
CAD gBlock	GTAGCCGAATGCTCTGTGcccAAGAAACCAATGGTGTTTAACGcccAAAAGGGATCACCCAGAATTTGTGCAATTGATTGTGGTTTGAAcccACTGAATCAGATAAAATGTTTTGTTTCACGTGGAGCTcccCGTGTTGAACTTGTGCCCTcccGGAATCATAAATTGGATGAGAAAcccCAATTTGATGGTTTATTCATAAGTAATGGACC		

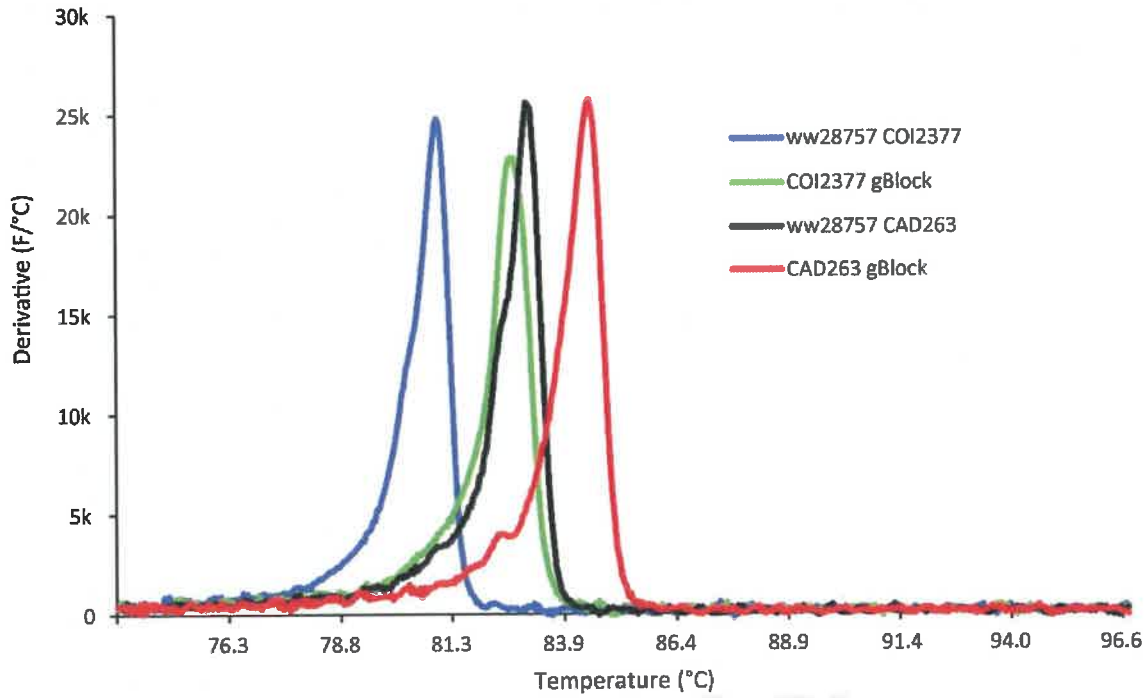
Table 2. Leafminer species tested for both COI2377 and CAD263 LAMP assays. Refer to Supplementary Table S1 for detailed specimen information.

Species	Family	Samples	Collected from Hosts	State
Liriomyza huidobrensis (Blanchard, 1926)	Agromyzidae	184	*Amaranthus* sp., *Phaseolus vulgaris*, *Hibiscus trionum*, *Brassica* sp., *Capsicum annuum*, *Apium graveolens*, *Stellaria media*, *Cucumis sativus*, Asteraceae sp., *Vicia faba*, *Lactuca sativa*, *Beta vulgaris*, *Sonchus* sp., *Spinacia oleracea*, *Solanum lycopersicum*, *Trifolium repens*, *Brassica rapa* , *Cucurbita pepo*	NSW, QLD
Liriomyza brassicae (Riley, 1885)	Agromyzidae	13	*Brassica* sp., *Brassica juncea*, *Sonchus* sp.	NSW, QLD
Liriomyza chenopodii (Watt, 1924)	Agromyzidae	8	*Spinacia oleracea*	NSW
Liriomyza sativae (Blanchard, 1938)	Agromyzidae	5	*Macroptilium atropurpureum*	QLD
Liriomyza trifolii (Burgess, 1880)	Agromyzidae	26	*Helianthus annuus* and laboratory colony	NT, WA
Liriomyza sp.	Agromyzidae	1	*Chenopodium album*	QLD
Calycomyza lantanae (Frick, 1956)	Agromyzidae	4	*Lantana camara*	QLD
Calycomyza humeralis (Roser, 1840)	Agromyzidae	6	*Rumex crispus*, *Erigeron* sp.	QLD
Chromatomyia syngenesiae (Hardy 1849)	Agromyzidae	46	*Glebionis coronaria*, *Leucanthemum sp*, *Sonchus* sp.	NSW, QLD
Phytomyza plantaginis (Goureau, 1851)	Agromyzidae	3	*Plantago major*	NSW
Scaptomyza australis (Malloch, 1923)	Drosophilidae	13	*Brassica* sp., *Brassica juncea and Spinacia oleracea*	QLD, NSW
Scaptomyza flava (Fallen, 1823)	Drosophilidae	1	*Sonchus* sp.	NSW
Tropicomyia polyphyta (Kleinschmidt, 1961)	Agromyzidae	4	*Lilium lancifolium*, *Araujia sericifera*	QLD
sp. indet. #01	Chloropidae	1	*Brassica juncea*	QLD
sp. indet. #02	Agromyzidae	4	*Trifolium* sp.	QLD
sp. indet. #03	Chloropidae	11	*Spinacia oleracea*	NSW

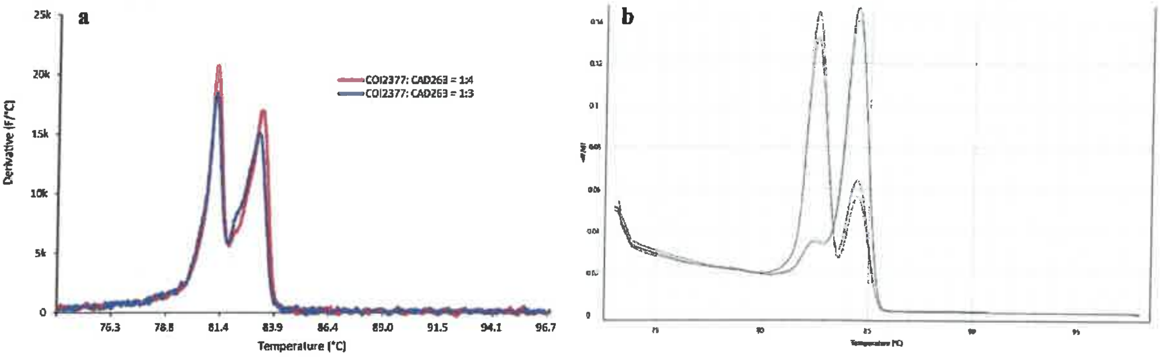

F3
F2
Floop
L. huidobrensis
L. trifolii
L. sativae
L. chinensis
L. brassicae
L. bryoniae
L. langei
F1
B1
Bloop
B2
B3

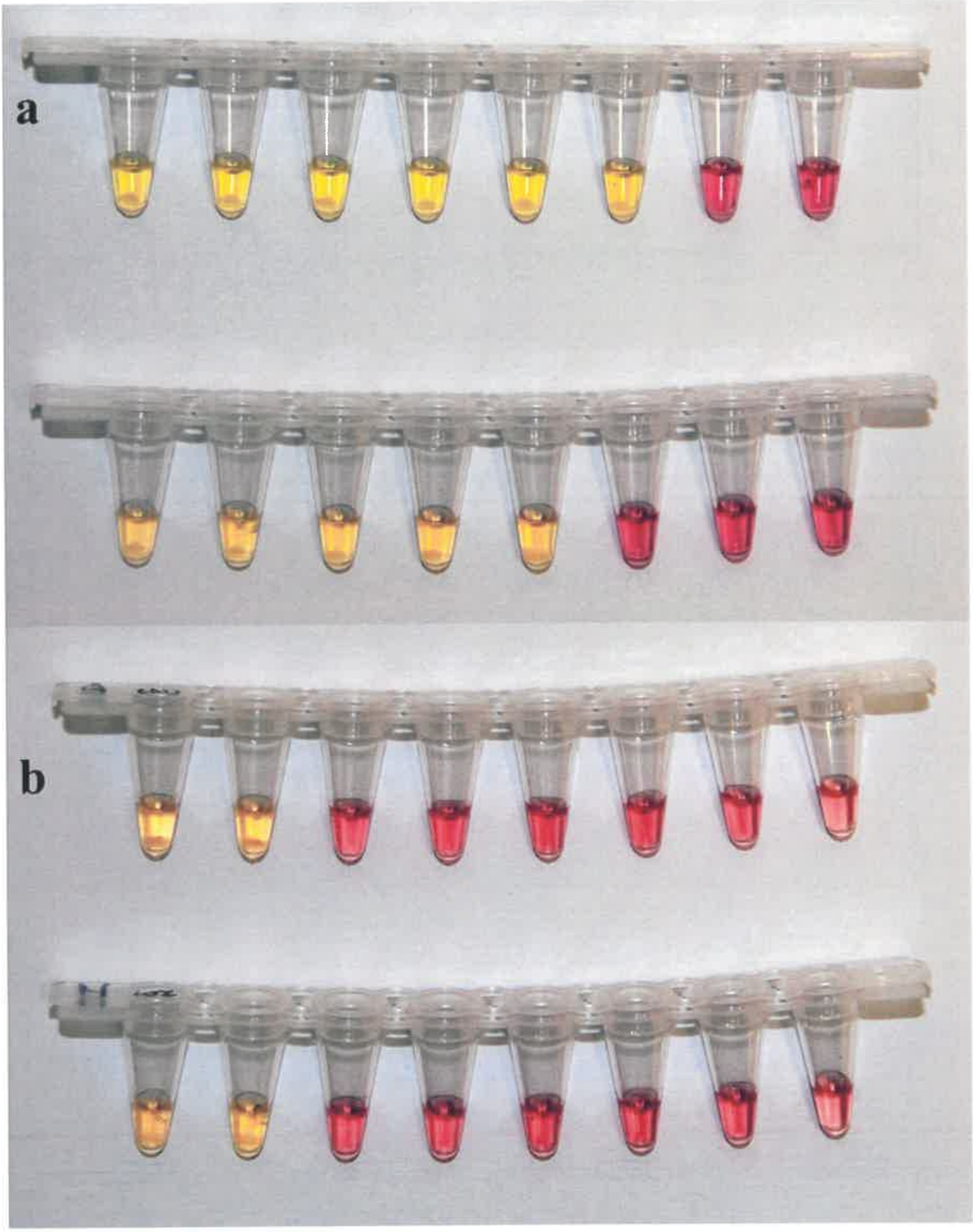

F3
F2
Floop
F1
B1
Bloop
B2
B3
L. huidobrensis
L. asclepiadis
L. baptisiae
L. brassicae
L. chinensis
L. cicerina
L. eupatorii
L. fricki
L. philadelphivora
L. trifoliearum
L. trifolii


a
b
Fluorescence
Time (mins)
Time (mins)
gBlock 10



30k
25k
20k
15k
10k
5k
0
Derivative (F/°C)
ww28757 COI2377
COI2377 gBlock
ww28757 CAD263
CAD263 gBlock
76.3
78.8
81.3
83.9
86.4
88.9
91.4
94.0
96.6
Temperature (°C)


a
COI2377: CAD263 = 1:4
COI2377: CAD263 = 1:3
Derivative (F/°C)
25k
20k
15k
10k
5k
0
76.3
78.8
81.4
83.9
86.4
89.0
91.5
94.1
96.7
Temperature (°C)
b


a
b

Two independent LAMP Assays for rapid identification of the serpentine leafminer, *Liriomyza huidobrensis* (Blanchard 1926) (Diptera: Agromyzidae) in Australia

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Supplementary Materials

Supplementary Methods S1. DNA barcoding methods

Leafminer specimens used in DNA barcoding were registered at the Barcode of Life Data systems (BOLD: http://www.boldsystems.org/). Specimen sampling details and associated specimen DNA barcodes & other gene sequences are available as a downloadable dataset "SLM and leafminers Australia" (DS-SLMWW), released at BOLD. Sequences were submitted via BOLD to the National Centre for Biotechnology Information (NCBI) and released with GenBank accession numbers (refer Supp. Table S1 for BOLD specimen records and associated GenBank accession numbers).

Specimen DNA extraction was preceded by a non-destructive tissue digestion. Whole leafminer fly specimens preserved in ethanol (90-95%) were transferred from collection vials into fresh microtubes allotted with individual alpha-numeric sample identifiers (ID) and incubated for half hours at room temperature to remove residual ethanol. Dried specimens were individually digested at 56° C overnight in 250 µL of DXT tissue digestion buffer (QIAGEN, Doncaster, Australia) incorporating 1% Proteinase K additive (QIAGEN). Following digestion, specimens were removed to new tubes containing 1mL of 90-95% ethanol for specimen curation at Biosecurity Collections Unit, Orange Agricultural Institute (NSW Dept. of Primary Industries), NSW Australia

DNA was extracted from 240 µL of each specimen digest using a KingFisher Flex robot and associated MagMAX CORE Nucleic Acid purification kits (Applied Biosystems). In some instances, speciemens were extracted manually using DNeasy Blood and tissue Kit (Qiagen). Final DNA eluted to 120 µL and were stored at -20° C.

Polymerase chain reactions (PCR) were prepared to a final volume of 15 µL using a MYRA Robotic Liquid Handling System (Bio Molecular Systems, Australia). PCR for DNA barcoding included 4 µL of DNA extract from single specimens, in the presence of Thermo Fischer Scientific reagents: 1X buffer, 2.8 mM MgCl2, 0.4 units of Platinum® Taq polymerase (Invitrogen), 200 µM dNTPs, and including 2 µM each of forward and reverse oligo-nucleotide primers (primers & primer combinations reported in Supplementary Table S2). Thermal cycling was completed using an Eppendorf Mastercycler ep gradient S PCR machine set with a universal thermal profile for all primer combinations: 95° C for 2 min; 40 cycles of 94° C for 30 s, 50° C for 30 s, 72° C for 45 s; 72° C for 5 min; storage at 4° C. PCR products stained with SYBR^{TM} Safe (Invitrogen) were visualized on a BioRad UV transilluminator after electrophoresis through a 1.5% agarose gel in 1% TAE buffer. Stained PCR products were qualitatively checked for expected fragment size against E-Gel 100bp ladder size marker (Invitrogen). PCR products sent to the Australian Genome Research Facility (Brisbane) were purified and bidirectional Sanger sequenced through an Applied Biosystems DNA Analyzer. Bidirectional sequence chromatograms were quality checked and assembled to sample ID using Lasergene SeqMan Pro ver. 8.1.0(3) (DNASTAR Inc., Maddison, WI, USA). Primer truncated sequences were aligned using BioEdit ver. 7.0.9.0[35]. All sequences were queried for species identity against publicly released sequence accession, using online engines: “Specimen Identification System” and “BLAST” respectively available at BOLD and GenBank. BOLD-allocated Barcode Index number (BIN) tagged to terminal clusters of similar DNA barcodes were auto-allocated at BOLD, to all compliant 5’ COI DNA barcodes[36].

Supplementary Table S2.

Oligo-nucleotide primers and anneal direction in PCR amplification of Leafminer mitochondrial COI 5’, COI 3’ and Nuclear CAD gene region sequences. Primers include 5’ forward or reverse M13 sequence tails (upper case) and gene specific sequence (lower case). Source references of primers as indicated. M13 tails reported here are as currently used at AGRF, differ marginally from tails reported in several of the source references.

Target	primer	Direction	Sequence (5′ – 3′)	References
COI 5'	*BC1Fm*	F	TGTAAAACGACGGCCAGTtcwacwaaycayaargayatygg	Folmer, et al. [37]
COI 3'	*SCOI C1-J-2183-fm*	F	TGTAAAACGACGGCCAGTcaacatttatttgatttttgg	Simon, et al. [38]
COI 5'	*LR1m*	F	CAGGAAACAGCTATGACCtaaacttctggatgtccaaaaaatca	Hebert, et al. [39]
COI 3'	*SCOI TL2-N-3014-rm*	R	CAGGAAACAGCTATGACCtccaatgcactaatctgccatatta	Simon, et al. [38]
CAD	Lir-CAD-53Fm	F	TGTAAAACGACGGCCAGTatgagaaagatgaatatggyatgcc	Carapelli, et al. [40]
CAD	Lir-CAD-689Rm	R	CAGGAAACAGCTATGACCtgrccrcgattaccaaatttcat	Carapelli, et al. [40]

References

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36 Ratnasingham, S. & Hebert, P. D. A DNA-based registry for all animal species: the barcode index number (BIN) system. *PLoS One* **8**, e66213 (2013).

37 Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294-299 (1994).
38 Simon, C. *et al.* Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Ann. Entomol. Soc. Am.* **87**, 651-701 (1994).
39 Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 14812-14817 (2004).
40 Carapelli, A. *et al.* Cryptic Diversity Hidden within the Leafminer Genus *Liriomyza* (Diptera: Agromyzidae). *Genes (Basel)* **9** (2018).

Cesar Australia

Appendix 2.1

Monitoring to support integrated pest management of *Liriomyza* spp. pests in Australia

A mini-review of global monitoring plans

Dr. Elia Pirtle

3 June 2022

Background

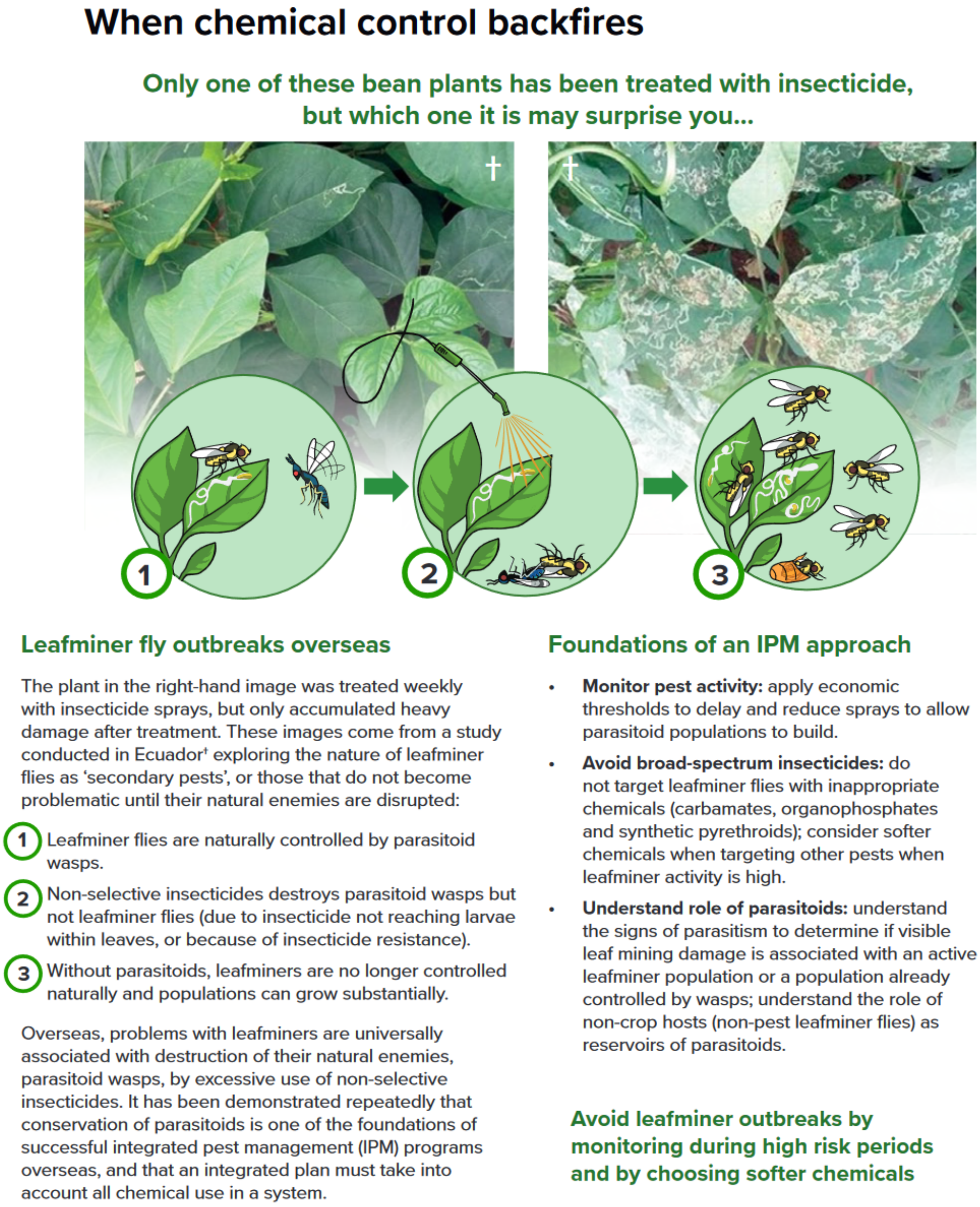
Three species of "leafminer flies" which have long been on the Australian federal government's 40 'high risk' biosecurity species, finally established in Australia between 2015 and 2020. They include the vegetable leafminer (VLM, *Liriomyza sativae*), the American serpentine leafminer (ASLM, *Liriomyza trifolii*) and the serpentine leafminer (SLM, *Liriomyza huidobrensis*). In 2008, VLM was detected for the first time throughout the north Australian islands of Torres Strait, and then on the Australian mainland at Seisia in 2015 (IPCC 2017). The pest has not yet been detected in any other regions of Australia despite ongoing surveillance efforts. Then in late 2020, SLM was detected in the Sydney region and eradication was subsequently deemed unfeasible (IPCC 2021a). Early the next year, ASLM was detected in northern Western Australia and within the Torres Strait, and final considerations on technical feasibility of eradication are still underway (IPCC 2021b), but eradication is unlikely.

> Read more about the recent SLM incursion here:
> https://cesaraustralia.com/pestfacts/serpentine-leafminer-detected-in-australia/

Referred to generally as the polyphagous *Liriomyza* leafminer, these flies are part of a well-known group (family Agromyzidae) of small, morphologically similar flies whose larvae feed internally on plants, often as leaf and stem miners. The majority of damage caused by polyphagous *Liriomyza* leafminer occurs during larval feeding between the upper and lower leaf surface, which curtails photosynthetic ability and reduces marketability of some crops.

Managing polyphagous leafminer

Global experiences support the notion that polyphagous Liriomyza leafminer are secondary pests, only reaching damaging levels after severe reductions in parasitoid populations. Polyphagous Liriomyza leafminer are also prone to evolving insecticide resistance, making control and eradication difficult. The most effective natural control of these pests comes from parasitoid wasps, but insecticide-based control disrupts beneficial predators and parasitoids, leading to secondary outbreaks.



The foundations of integrated pest management for exotic polyphagous *Liriomyza*. Image source: Chirinos, DT., Castro, R., and Garces, A. (2017). Read more about leafminer management here: https://ausveg.com.au/app/uploads/2020/12/Management-Plan-Exotic-leafminers.pdf

Monitoring is a cornerstone of a successful IPM approach to managing the polyphagous *Liriomyza* leafminer. As reviewed in Ridland et al (2020): "Successful field programs to manage a spectrum of insect pests including *L. sativae* and *L. trifolii* have been implemented for tomato and celery in California (Johnson et al. 1980a, 1980b, 1980c; Trumble 1985; Reitz et al. 1999), watermelon in Hawaii (Johnson 1987, 2005; Johnson et al. 1989) and melon and lettuce in Arizona (Palumbo & Kerns 1998; Palumbo & Castle 2009).The foundations of these programs are to (1) reduce initial leafminer pressure by using uninfested transplants, destroying weeds and deep ploughing of senescent crops and avoiding planting new crops adjacent to old crops (Capinera 2017) and (2) conserve parasitoid wasps by avoiding broad-spectrum insecticides (Johnson et al. 1980b; Trumble & Toscano 1983) and using economic thresholds to delay and reduce sprays to allow colonising parasitoid populations to build up".

Monitoring goals as part of IPM programs may include:

1. Detecting early infestations, particularly in young crops or high value, zero-tolerance crops for which leaf mine damage reduces marketability, such as ornamentals, lettuce and celery;
2. Estimating population density in larger infestations in fruiting field crops, such as tomato and potato, in order to apply economic thresholds to chemical applications and to monitor the success of interventions (we focus here for the rest of this article). Sampling techniques aimed at estimating population density to support the use of ETs include:
    a. Counts of infested leaves
    b. Counts of live *Liriomyza* larvae within leaf mines (aided by a hand lens)
    c. Counts of *Liriomyza* pupae (caught in 'pupal trays' or rearing bags)
    d. Counts of *Liriomyza* adults on yellow sticky traps

Each technique has benefits/drawbacks for each of the monitoring goals discussed listed above and can be used in combination to effectively monitor populations of *Liriomyza* spp. pests in Australia.

Counts of infested leaves

Searching for leaf mines present on leaves is the simplest way to gauge the presence and activity of leafminer flies and some sampling plans have been developed that rely on count of leaf mines, without further confirmation of the presence of living larvae (which often requires a hand lens) (Burgio et al., 2005). These plans are usually based on counting the number of leaves bearing leaf mines (see Figure 1) in a subset of leaves on a subset of randomly selected plants.

However, a confounding factor for these plans is that the detection of mines does not always indicate active populations of flies (particularly in longer lifespan fruiting crops), as the mines persist on the leaf long after the emergence of the fly larva. Visual damage alone can be difficult to relate to active population size, as a result of the accumulation of older damage through time and the difficulty of detecting live larvae inside mines (Heinz & Chaney, 1995). In a worst-case scenario, inflated estimates of active population sizes may influence growers to spray unnecessary chemicals onto crops where leafminer populations have already collapsed, due to environmental factors or the influence of beneficial insects. In these cases, more harm is done than good if beneficial parasitoids are destroyed, allowing the pest population to flourish once again (Ridland et al., 2020).

Pro: easy to see leaf mines and stippling damage without a hand lens

Con: can overestimate population activity and encourage inappropriate interventions

In Summary: Preferred when the goal is to detect early infestations or to monitor infestations in short lifespan crops, but may be inappropriate for monitoring infestation in long lifespan crops, or for monitoring the success of an intervention

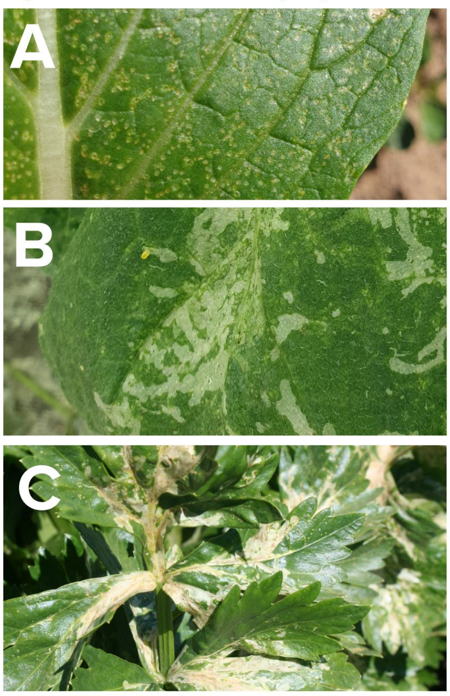


Figure 1: Damage caused by adult leafminer. A) SLM stippling damage to choy sum (Shannon Mulholland, NSW DPI); B) SLM damage to cucumber (Shannon Mulholland, NSW DPI); C) SLM damage to celery (John Duff, DAFF)

Counts of live larvae:

Counting larvae within leaf mines is more difficult than simply observing (or counting) leaf mines, as it generally requires the use of a hand lens to carefully check the wider ends of mines for a small whitish-yellow larva (see Figure 2), and for best results requires that living larvae can be distinguished from dead larvae. However, this method can produce significantly more accurate results for estimating population sizes, especially in longer lifespan crops like tomato, which can accumulate more damage before the plants are adversely affected. This method is most suited to supporting the use of economic thresholds, and monitoring the success of interventions. Sampling plans based on larval counts are usually based on counting the number of 'active' mines in a subset of leaves on a subset of randomly selected plants, by checking mines for live larvae using a hand lens.

Counting larvae within leaf mines is the most labour intensive method, but also the most accurate method, having two major advantages over the use of traps such as pupal trays and yellow sticky traps: 1) it is most directly related to damage potential assessment as it focuses on the life stage responsible for the majority of damage; and 2) the resulting data is easier to incorporate directly into a decision making program that is based on population presence and allows for pesticide efficacy to be evaluated post sprays (Namvar et al., 2012).

Pros: accurate measure of population density, accounts for idiobiont ectoparasitoid activity (see Figure 5 and the "Monitoring for beneficial wasps" breakout box)

Cons: requires a hand lens and close inspection of leaves, underestimates koinobiont endoparasitoid activity (see Figure 5 and the "Monitoring for beneficial wasps" breakout box)

In Summary: preferred when monitoring infestation in long lifespan crops, or for monitoring the success of an intervention as it gives the most accurate population size estimates and is therefore a key component of global sampling plans aimed at using economic thresholds (ETs)

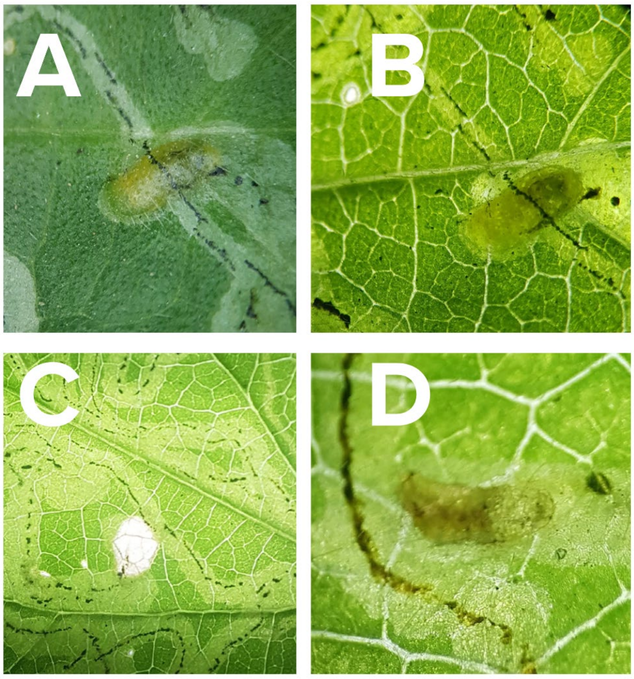


Figure 2. A) Live larvae (VLM pictured) can be seen feeding via a hand lens; B) Holding the leaf up to the sun can increase visibility of larvae inside mines; C) Inactive mines may be empty; or D) may contain a dead larva. (Elia Pirtle, Cesar Australia)

Counts of pupae:

PUPAL TRAYS

Johnson, Oatman, & Wyman (1980) described a method for monitoring leafminer in fresh market tomatoes based on counts of pupae collected within pupal trays (see Figure 3). The study showed that the number of pupae collected in pupal trays correlated significantly with the number of live larvae within leaflets. Thus, pupal tray sampling was efficient, inexpensive and more sensitive to population size changes than leaflet sampling (focusing on counting larvae within leaflets), and the trays became an integral part of an IPM program implemented for fresh market tomatoes in California. According to their method, Leafminer activity can be measured by collecting mature larvae which have fallen into polystyrene or plastic trays (pupal trays, between 8 x 11 to 12 x 15 inches in size) and pupated over a period of 3-4 days. These styrofoam trays are placed on the ground underneath plants and left in place for three days, at which point pupae trapped within the trays are counted, then removed and the traps replaced for further counts.

Pro: accounts for idiobiont ectoparasitoid activity (Figure 5), preferred as an alternative to counting live larvae in long lifespan fruiting crops such as tomato, as it does not require a hand lens as pupae are easier to observe and count after emergence

Cons: underestimates koinobiont endoparasitoid activity unless samples are retained for several weeks for rearing (Figure 5), can be poorly suited to short, leafy, or densely clumped crops such as lettuces and celery, can be poorly suited to wet areas

In Summary: Pupal trays are a popular method in long lifespan fruiting crops overseas due to being an easily visual indicator of whether leaf mine damage is caused by an active infestation, or whether the damage is old and thus intervention may be unwarranted; gives accurate population size estimates and can be used with Economic Thresholds.)

LEAF COLLECTION AND REARING

Pupae may also be counted by collecting a subset of leaves from a subset of randomly selected plants into plastic bags and observing the number of pupae that emerge and collect into the bottom of the bag (see Figure 3). This method has been incorporated into sampling plans such as in Foster (1986) to reduce reliance upon hand lens inspection of mines. Moreover, the pupae collected via pupal trays or via leaf collections may be retained in order to assess the level of parasitism by koinobiont endoparasitoids. Pupae may be kept in a plastic bag with a damp paper towel, out of direct sunlight, until adult flies or wasps emerge and adult flies may be counted. This improves accuracy of leafminer population size estimates because it accounts for accounts for idiobiont ectoparasitoid and koinobiont endoparasitoid activity. However, it can take multiple weeks for all adult flies to emerge and wasps even longer, and is thus not suitable for quick decisions.

Pro: accounts for idiobiont ectoparasitoid activity (Figure 5), does not require a hand lens as pupae are easier to observe and count after emergence

Cons: underestimates koinobiont endoparasitoid activity unless samples are retained for several weeks for rearing (Figure 5),

In Summary: Colleting leaf samples for rearing provides clear visual indicators of whether leaf mine damage is caused by an active infestation, or whether the damage is old and thus intervention may be unwarranted; gives accurate population size estimates and can be used with Economic Thresholds.

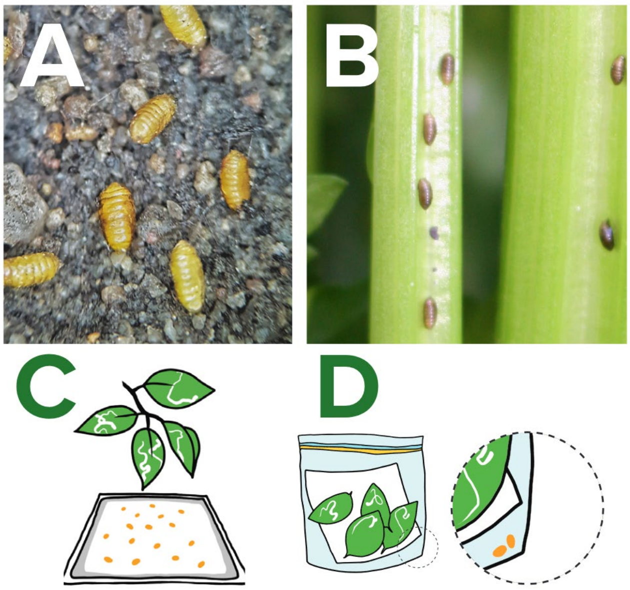


Figure 3. Small orange pupae (~2mm; VLM pictured) accumulate in the soil beneath infested plants (Elia Pirtle, Cesar Australia). B) SLM pupae collecting on plant surfaces in celery (John Duff, DAFF); Pupae can be collected into C) pupal trays placed underneath plants; or D) into the bottom of plastic bags on leaf collections.

Counts of adults:

Agromyzid flies are attracted to the colour yellow, and can therefore be captured on yellow sticky traps (see Figure 4), which are used to monitor a variety of invertebrate pests. Yellow sticky traps have been shown to be more effective for *Liriomyza* adults than other types of traps, such as funnel traps and yellow water pans, and vacuum sampling (Chavez & Raman, 1987; Weintraub, 2001).

A great deal of effort overseas has been dedicated to improving the effectiveness of yellow sticky traps for *Liriomyza* adults including the modifications of size, shape, adhesives, lures, height and orientation. For example, several studies report a strong effect of trap height on the number and species trapped, however these results do not always appear consistent and may be difficult to extrapolate across different crop types. Moreover, optimal height may vary considerable between *Liriomyza* species (Zehnder & Trumble, 1984). Sticky traps make for good indicators of leafminer presence and can be used to monitor movements of populations throughout or between paddocks, or indicate times of migration into a crop (Palumbo & Kerns, 1998). Sticky traps do have a few additional shortcomings, including (1) sticky traps require visual searches and rough morphological identifications must be made, (2) sticky traps appear to be are poor indicators of leafminer population sizes (sources) and are thus difficult to relate to damage and (3) sticky traps are poor indicators of parasitoid activity (Weintraub, 2001).

Experimental lures developed from the extracted volatiles of known plant hosts have been shown to be attractive to *Liriomyza*. For example, lures made from spruce, basil, juniper or clove oil have been shown to attract serpentine leafminer (Gorski, 2005). However, there are no products commercially available for use on *Liriomyza*.

Pros: does not require a hand lens as pupae are easier to observe and count after emergence

Cons: difficult to relate to population sizes and damage levels,

In Summary: Popular method overseas due to being an easily visual indicator of whether leaf mine damage is caused by an active infestation, or whether the damage is old and thus intervention may be unwarranted; gives accurate population size estimates and can be used with Economic Thresholds.

Figure 4. A yellow sticky trap hung above a tomato plant (left) and an adult VLM captured on the trap (right). (Elia Pirtle, Cesar Australia)

Monitoring for beneficial wasps

Idiobiont parasitoids:

SLM larvae which have been attacked by idiobiont parasitoid wasps (Fig. 5a) are immediately paralysed and never emerge from the leaf mine. Thus, counting *living* larvae (i.e. those actively feeding inside leaf mines) or pupae that have emerged from leaves avoids counting any larvae that were already attacked by idiobiont wasps, which would inflate the SLM population size estimate. Idiobiont ectoparasitoids* (which develop outside the body of the fly) can be observed inside leaf mines through a hand lens as either a larva, often found in close proximity to a dead leafminer larva (Fig. 5b), or as a pupa, flanked by black dots called meconial pillars (Fig. 5c).

In summary: Signs of idiobionts can be observed inside leaf mines via a hand lens (Fig. 5b/c)

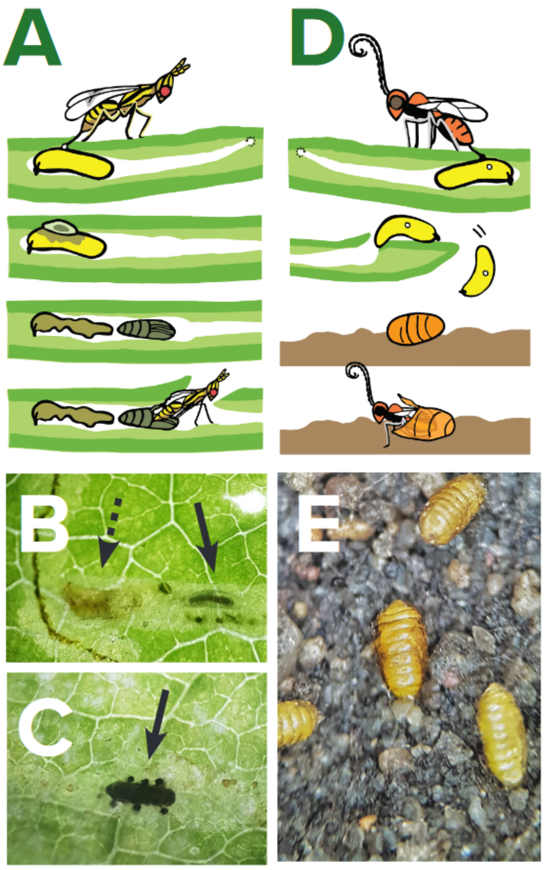
Koinobiont parasitoids:

SLM larvae which have been attacked by koinobiont wasps are NOT paralysed. They continue feeding and successfully pupate. Thus, counting living larvae or pupae that have emerged from leaves may still inflate the SLM population size estimates, because these counts may include individuals that will ultimately perish during the pupal stage. Koinobiont endoparasitoids (which develop inside the body of the fly) emerge from otherwise healthy looking leafminer pupae. Thus, pupae must be retained in rearing vessels for several weeks to confirm activity of these wasps.

In summary: There are no signs of koinobiont parasitism until emergence of adults from otherwise healthy looking fly pupae.

**some idiobionts are endoparasitoids, which pupate inside the dead fly larva, and thus may be less visible within the mine, however this is a minority of species*

Fig 5. Parasitoid lifecycles



A) An idiobiont ectoparasitoid lifecycle and signs of idiobiont parasitism under a microscope or hand lens, including B) a wasp larva (solid arrow) near a leafminer larva carcass (dotted arrow); and C) a wasp pupa inside a leaf mine (solid arrow). D) A koinobiont endoparasitoid lifecycle. E) There are no signs of koinobiont parasitism before emergence of adult flies and wasps from the fly puparium.

Getting an accurate population estimate

Making accurate estimates of leafminer populations is a prerequisite to using economic thresholds, aimed at reducing unnecessary chemical costs and unwanted toxicity effects on beneficials. However, leafminer distributions in a paddock are often clumped (as is true for many pests) which means that your population estimate may vary widely based on what part of the paddock you searched. However, you can use mathematical rules to tell you exactly how many plants you must search before you can be reasonably confident that your measured pop density captures enough variation to accurately reflect the whole paddock. In the case of these patchy distributions, Taylor's power law becomes an appropriate method for determining sample sizes (Ruesink, 1980).

Thus, several types of sampling plans, based on these mathematical rules for non random aggregations, have been developed and applied overseas to estimating leafminer populations (Burgio et al., 2005; Heinz & Chaney, 1995; Jones & Parrella, 1986; Namvar et al., 2012) for the purposes of making informed management decisions (Table 1). These can generally be split into 'conventional' and 'sequential' sampling plans.

- Conventional sampling plans operate on a fixed number of samples that are taken per unit of area, and the resulting precision of the population size estimate will vary with the population density (Lopes et al., 2019).
- Sequential sampling plans on the other hand have a pre-determined level of precision which must be reached, and samples are taken until that fixed level of precision is reached. The ultimate number of samples that must be taken relates to the population density, and surveyors know when sufficient samples have been collected by referring to a pre-calculated 'stop line' (see Figure 6 for an example).

Conventional sampling plans tend to be the starting points for developing decision making systems for pest control interventions (Lopes et al., 2019), while sequential sampling plans can provide increased efficiency (Namvar et al., 2012).

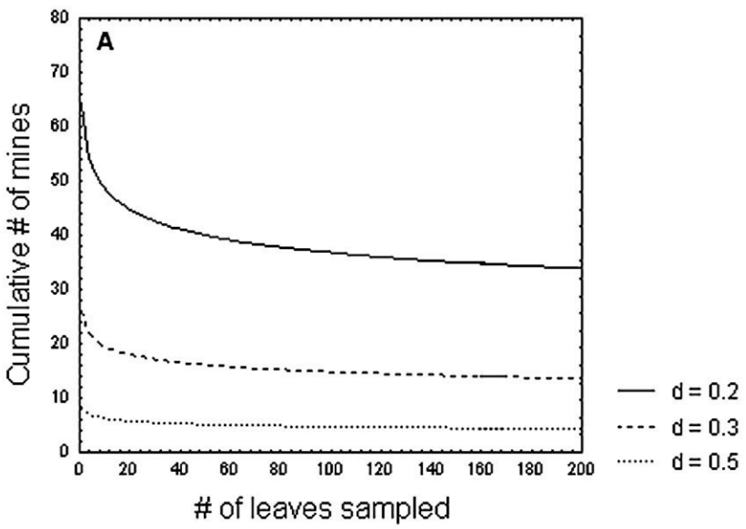


Figure 6. An example graph showing stop lines for leaf mine counts, reproduced from Burgio et al. (2005). Each line on the graph shows the "stop numbers" for three levels of precision, where you can stop counting once

you reach the desired number of mines per the number of leaves you have checked. To use the stop line, keep a cumulative tally of how many mines you have counted alongside how many leave you have checked, and stop counting once you reach the number of mines per leaf corresponding to a point on the line of your chosen accuracy level. For example, if after checking about 40 leaves you if you count more than 40 mines, you know you have done enough sampling to estimate population size with only a 20% margin of error.

Table 1 provides a summary of several conventional and sequential sampling plans for leafminer in a variety of crops, and provides key rule of thumbs from these plans. These plans may provide some rough rules of thumb that can serve as starting point in Australia, however, they cannot be relied upon as accurate sampling plans in Australia until they are formally validated. Australian specific sampling plans and economic thresholds will need to be created to support successful IPM programs to manage exotic *Liriomyza* spp. leafminer.

Table 1: Population density sampling plans implemented for *Liriomyza* species globally in commercial crops.

Leafminer species and crop	Reference	Reference Title	Type	Sample unit	Summary of plan	Other notes
SLM in tomato	(Lopes et al., 2019)	Practical sampling plan for *Liriomyza huidobrensis* (Diptera-Agromyzidae) in tomato crops	Conventional	Active mines (e.g. live larvae)	Count active mines in 73 leaf samples per field (irrespective of field size up to 10 ha), taking random leaves from the basal leaf of the middle section of the plant canopy	Average time requirement was 30 min of leaf evaluation time (plus walking time which was up to one hour for 10 ha fields)
SLM in potato	(Alves et al., 2014)	A Sampling Plan for *Liriomyza huidobrensis* (Diptera: Agromyzidae) on a Potato (*Solanum tuberosum*) Plantation	Conventional	Active mines (e.g. live larvae)	Count active mines in one random leaf sample from the middle canopy section from 15 random plants (at least 50m apart) per 24.5 ha	Average 30 minutes total sampling time per 24.5 ha Cost was significantly lower than insecticides
VLM in glasshouse cucumber	(Namvar et al., 2012)	Estimation of larval density of *Liriomyza sativae* Blanchard (Diptera Agromyzidae) in cucumber greenhouses using fixed precision sequential sampling plans	Sequential	Active mines (e.g. live larvae)	Count active mines per leaf in random leaf samples until a larvae count stop line (based on desired level of accuracy) is reached (See Supp Fig 1).	With the precision of 0.28, samples required varied between 2 to 157 leaves, when mean larval density per leaf declined from 29.1 to 0.07. For precision of 0.25, densities > 4 larvae per leaf required < 11 samples, but densities of < 1 larvae required > 32 samples
VLM in glasshouse cucumber	(Namvar et al., 2011)	Fixed precision sequential sampling plans for leaf mines of Liriomyza sativae	Sequential	Active mines (e.g. live larvae)	Count active mines per leaf in random leaf samples until a larvae count stop line (based	Sample sizes ranged from 3 to 197 and 15 to 1229 leaves at the precision levels of 0.25 and 0.1 respectively.

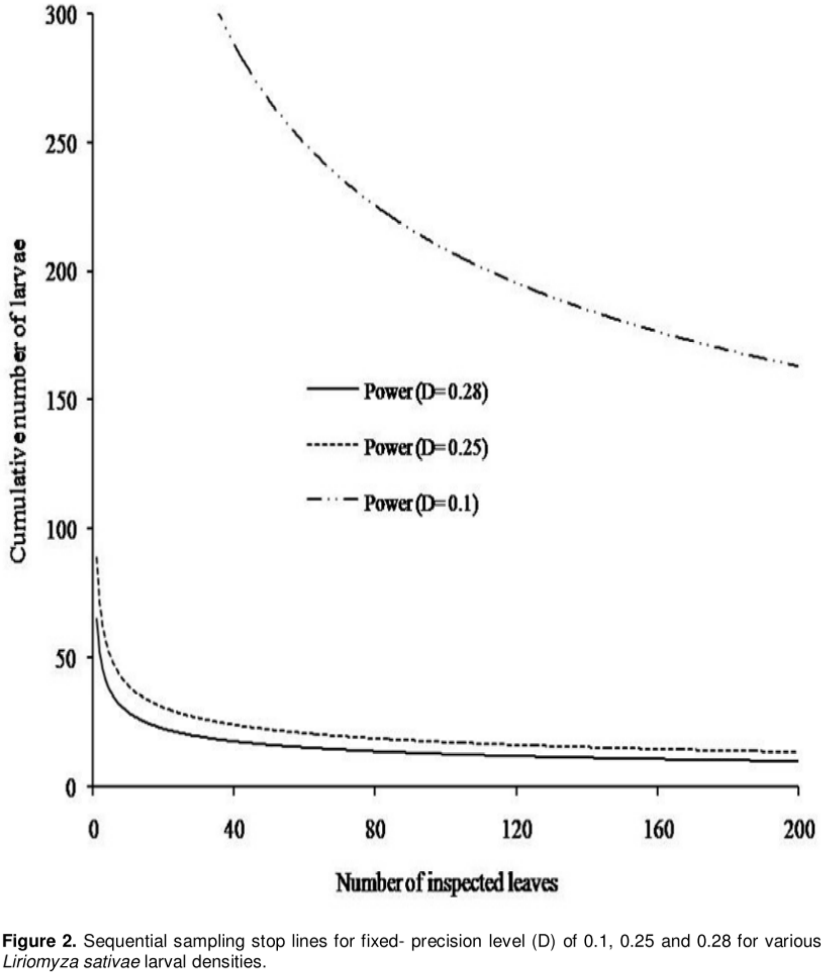
		Blanchard (Diptera: Agromyzidae) in cucumber greenhouses			on desired level of accuracy) is reached.	This is an earlier analysis of the data used within Namvar er al. (2012)
LM in tomato	(Schuster & Beck, 1992)	Presence-absence sampling for assessing densities of larval leafminers in field-grown tomatoes	Presence-Absence	Proportion of leaflets that contain active mines	Record the proportion of leaflets that have any live larvae present by checking the upper surface of the terminal three leaflets of the 7th leaf from the top of either a main stem, lateral or sub-lateral stem from randomly selected plants.	Proportion infested leaves can be used to predict number of larvae present per sample, to reduce counting time per leaflet This study did not address how many samples needed to create an accurate paddock wide density estimate
SLM in lettuce	(Burgio et al., 2005)	Spatial Patterns and Sampling Plan for *Liriomyza huidobrensis* (Diptera: Agromyzidae) and Related Parasitoids on Lettuce	Sequential	Mined leaves (not distinguishing active from inactive mines)	Count leaves with mines from random leaf samples until the number of mined leaves collected exceed stop line values for the number of overall leaves collected (See Supp Fig 2).	This paper advises that damage thresholds cannot be predetermined as they may vary by environment/agroeconomic conditions
SLM in celery	(Heinz & Chaney, 1995)	Sampling for *Liriomyza* *huidobrensis* (Diptera: Agromyzidae) larvae and damage in celery	Sequential	Active mines (e.g. live larvae)	Count all active mines per randomly selected plants until a larvae count stop line (based on desired level of accuracy) is reached (See Supp Fig 3), with a possible maximum sample size of 100 petioles	Sequential sampling plan accurately estimates mean densities > 17.5 live larvae per 100 petioles with a 0.25 level of precision Lower densities of larvae or mines required sample sizes > 100 petioles at a level of precision ≥ 0.25 to accurately estimate cumulative or mean leafminer densities.

						Validation tests showed that using frequencies of infested petioles as a proxy for counting active mines overestimated population density
LM in watermelon	(Lynch & Johnson, 1987)	Stratified Sampling of *Liriomyza* spp. (Dipetra: Agromyzidae) and Associated Hymenopterous Parasites on Watermelon	Stratified	Active mines (e.g. live larvae) per leaf	Count larvae within medium sized leaves, randomly selected within the area greater than 0.5 meters from either end of the plant vine (because of higher variation in insect densities in the extreme basal and distal portions of a vine)	Standard errors were reduced by >46 and 35%, respectively, when leaf sizes were stratified (by dividing vines into 50 cm intervals, or strata, starting at the plant base and ending in the distal end of the vine, and taking random leaf samples within each strata) This study did not address how many samples needed to create an accurate paddock wide density estimate
ASLM in chrysanthemum	(Jones & Parrella, 1986)	Development of Sampling Strategies for Larvae of *Liriomyza trifolii* (Dipetra: Agromyzidae) in Chrysanthemums	Conventional	Active mines (e.g. live larvae)	Count active mines from three leaves per each randomly selected plant until 100 leaves have been samples.	After about 3 weeks, sampling should focus on the bottom strata of the plant, and after 6 weeks, sampling should focus on the middle strata of the plant (where larval numbers tend to be highest)
ASLM in celery	(Foster, 1986)	Monitoring Populations of *Liriomyza trifolii* (Diptera: Agromyzidae) in Celery with Pupal Counts	Conventional	Pupae emerging from picked leaves	Pick ten terminal leaflets from each of 10 randomly selected plants, at each of ten systematically placed sites within the paddock. Place leaflets into a plastic bag and maintain them for no more than ten days, and then count all emerged pupae.	As a rule of thumb, assume 5 pupa or less per 10 leaflet samples poses no economic threat Number of samples necessary depends on leafminer densities, where, if average density is >5 pupa per 10 leaflet samples, 10 sample sites (of 10 leaflets each) yields 25% level of precision

						The sampling plan required 30 to 45 minutes total time to sample an 11 hectare field
LM in tomato	(Zehnder & Trumble, 1985)	Sequential Sampling Plans with Fixed Levels of Precision for *Liriomyza* species (Diptera: Agromyzidae) in Fresh Market Tomatoes	Sequential	Adults on yellow sticky traps	Adults are counted on sticky traps until the cumulative number of adults exceeds the stop line value for the number of sticky traps checked (See Supp Fig 4).	Approximate number of sticky traps that must be place in a field to yield enough samples to reach the desired precision level can be estimated based on how many adults are caught on 'pilot' yellow sticky traps (see Supp Fig 5).
LM in tomato	(Zehnder & Trumble, 1985)	Sequential Sampling Plans with Fixed Levels of Precision for *Liriomyza* species (Diptera: Agromyzidae) in Fresh Market Tomatoes	Sequential	Pupae within pupal trays	Pupae counted within pupal trays until the cumulative number of pupae exceeds the stop line value for the number of pupal trays checked (See Supp Fig 4).	
ASLM in greenhouse chrysanthemum	(Parrella & Jones, 1985)	Yellow Traps as Monitoring Tools for *Liriomyza trifolii* (Diptera: Agromyzidae) in Chrysanthemum Greenhouses	Sequential	Adults on yellow sticky traps	Adults are counted on sticky traps until the cumulative number of adults exceeds the stop line value for the number of sticky traps checked (See Supp Fig 6).	Traps must be placed over 'homogenous' blocks of plants (planted less than 30 days apart) A validation trail showed only 18% of 792 traps that had been placed needed to be counted to provide sufficient accuracy for population size estimates.

[Insert table footnotes with the Cesar Caption style]

Supplementary Figures



Supplementary Figure 1. Stop lines for live larva counts, reproduced from Namvar et al. (2012).

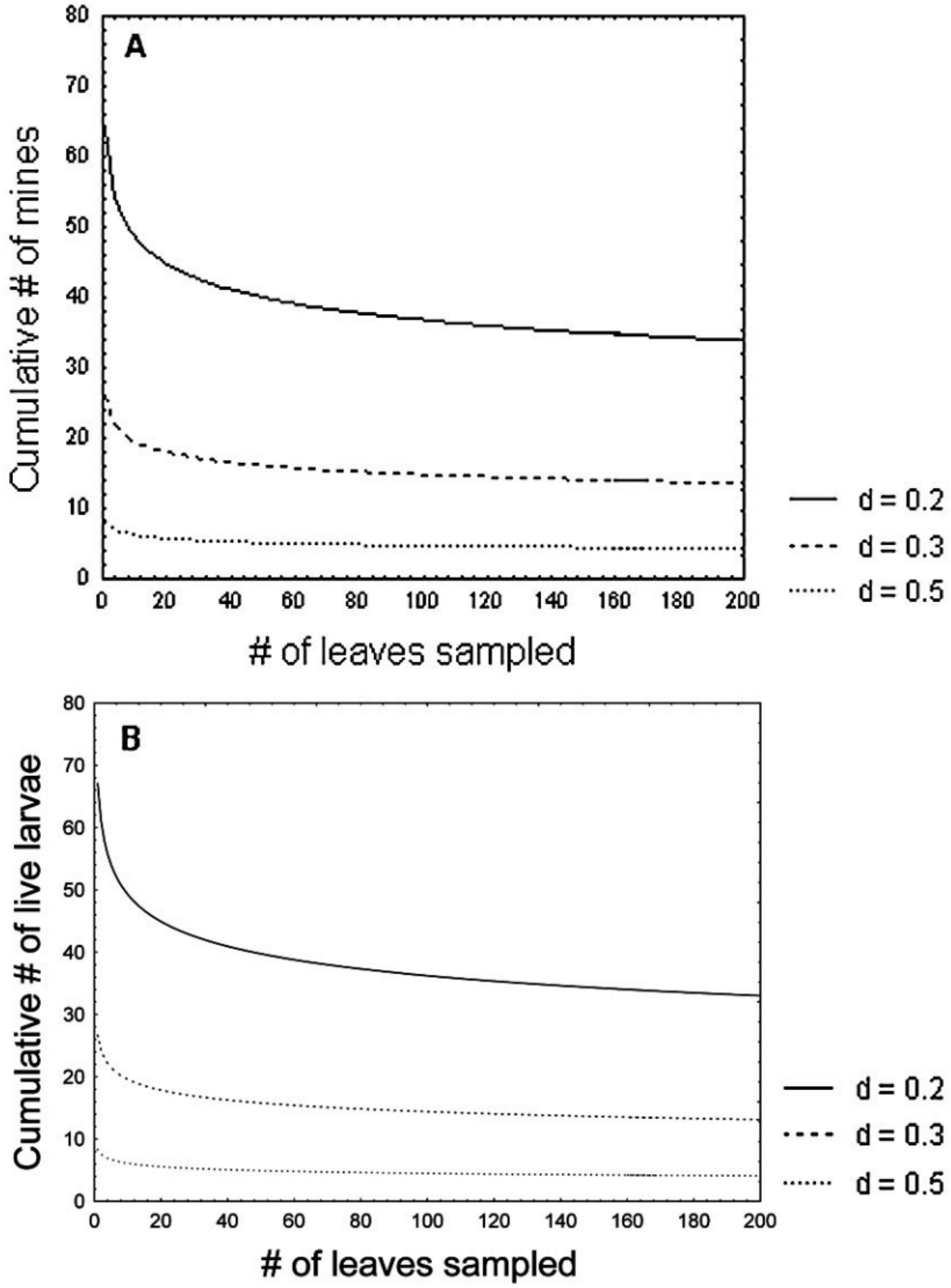


Fig. 1. Stop lines calculated for *L. huidobrensis* mines (A) and live larvae (B) at three different precision levels.

Supplementary Figure 2. Stop lines for live larva counts compared to mine counts, reproduced from Burgio et al. (2005)

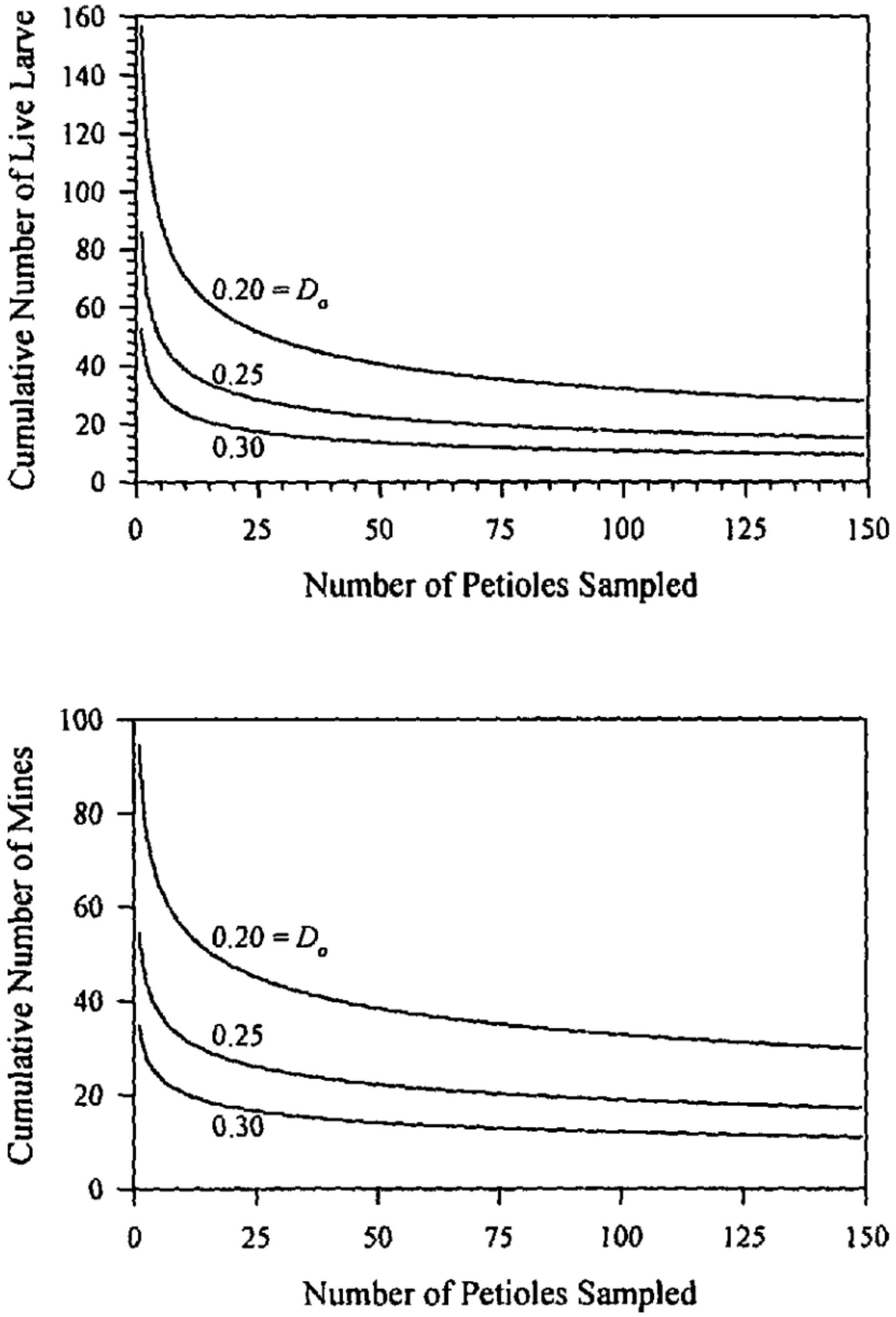


Fig. 3. Stoplines for constant-precision sequential samples for L. *huidobrensis* larvae (top) and mines (bottom) at three levels of precision (D_0) equal to 0.20, 0.25, and 0.30.

Supplementary Figure 3. Stop lines for live larva counts compared to mine counts, reproduced from Heinz & Chaney (1995)

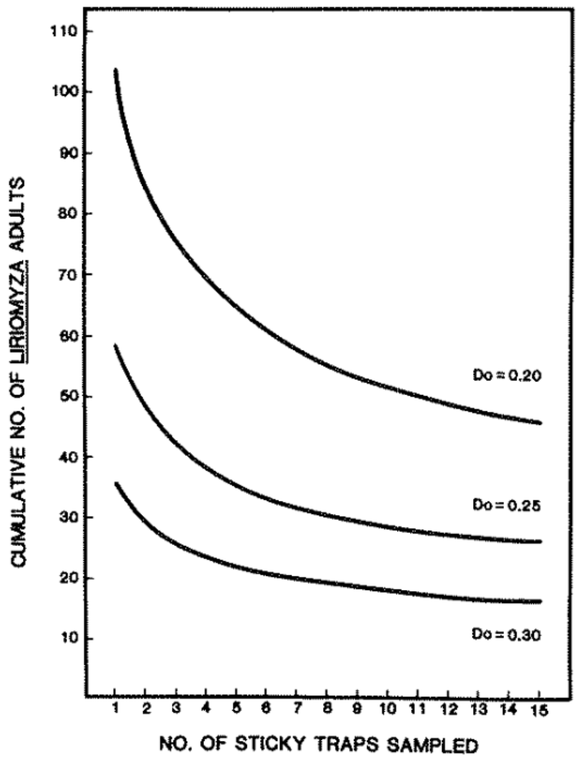


Fig. 2. Stoplines for constant-precision sequential samples for *Liriomyza* adults at three levels of precision (D_0) of 0.20, 0.25, and 0.30.

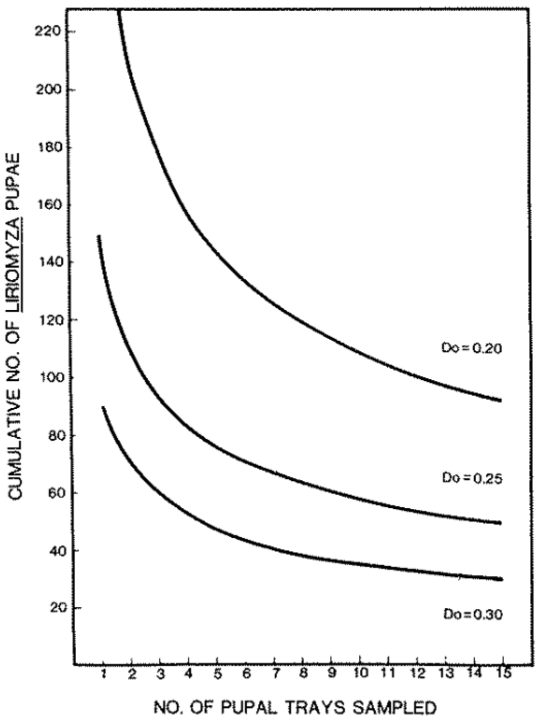


Fig. 3. Stoplines for constant-precision sequential samples for *Liriomyza* pupae at three levels of precision (D_0) of 0.20, 0.25, and 0.30.

Supplementary Figure 4. Stop lines for sticky trap and pupal tray samples, reproduced from Zehnder & Trumble (1985)

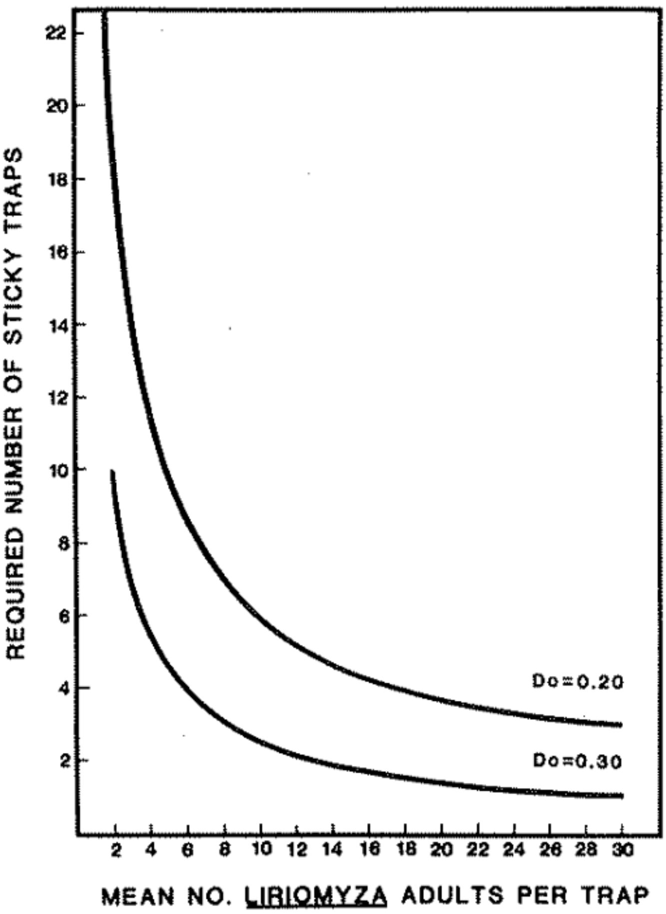


Fig. 4. Required number of sticky traps for various *Liriomyza* adult densities at two levels of precision (D_0) of 0.20, 0.30.

Supplementary Figure 5. A guideline for determining the approximate number of sticky traps that must be place in a field to yield enough samples to reach the desired precision level, based on how many adults are caught on ‘pilot’ yellow sticky traps, reproduced from Zehnder & Trumble (1985)

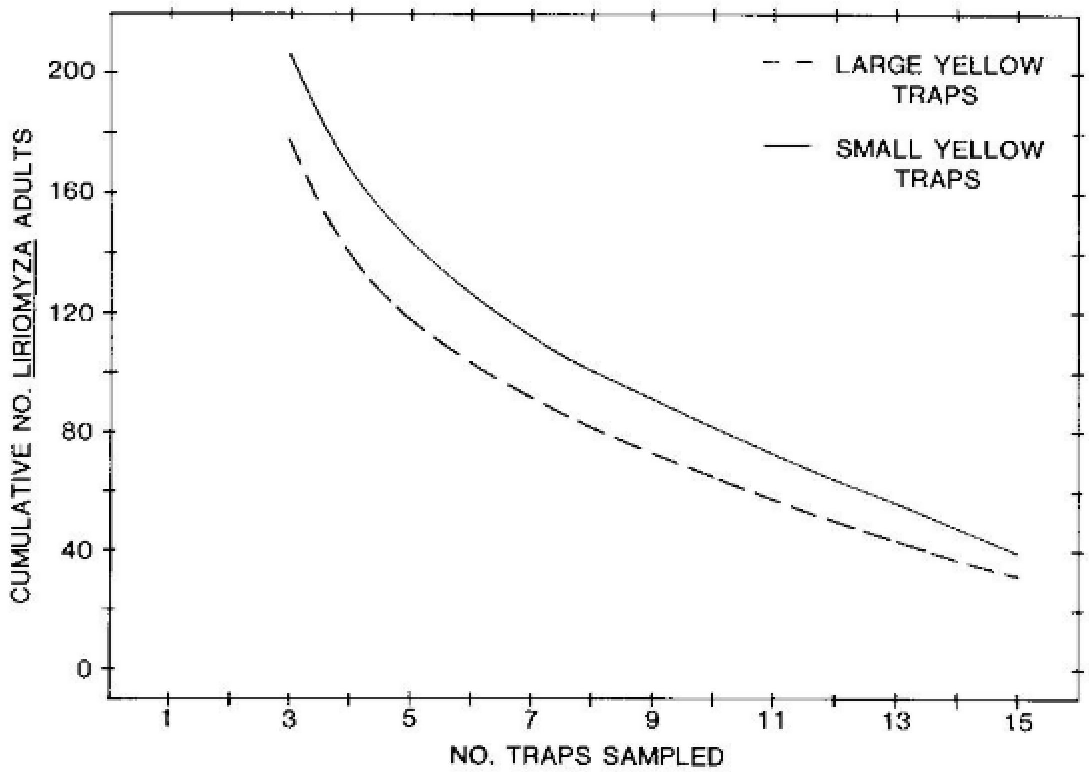


Fig. 3. Stoplines for constant precision sequential samples for large and small sticky cards used to trap *L. trifolii*, generated using Iwao's patchiness regression. The fixed level of precision is 0.25.

Supplementary Figure 6. Stop lines for sticky trap and pupal tray samples, reproduced from Parrella & Jones (1985)

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Monitoring for beneficial wasps

Idiobiont parasitoids:

SLM larvae which have been attacked by idiobiont parasitoid wasps (Fig. 5a) are immediately paralysed and never emerge from the leaf mine. Thus, counting living larvae (i.e. those actively feeding inside leaf mines) or pupae that have emerged from leaves avoids counting any larvae that were already attacked by idiobiont wasps, which would inflate the SLM population size estimate. Idiobiont ectoparasitoids* (which develop outside the body of the fly) can be observed inside leaf mines through a hand lens as either a larva, often found in close proximity to a dead leafminer larva (Fig. 5b), or as a pupa, flanked by black dots called meconial pillars (Fig. 5c).

In summary: Signs of idiobionts can be observed inside leaf mines via a hand lens (Fig. 5b/c)

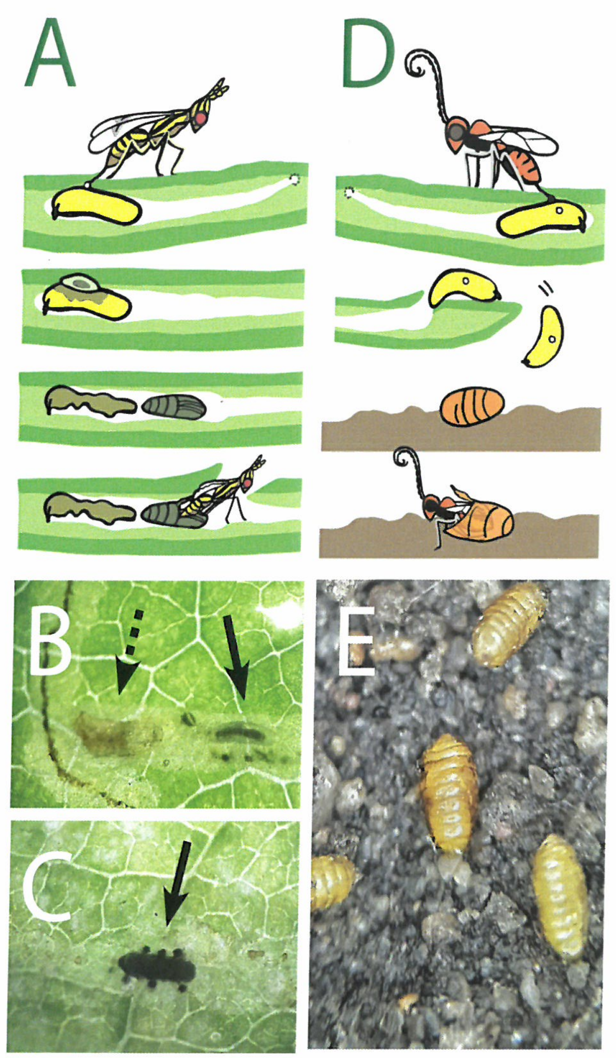
Koinobiont parasitoids:

SLM larvae which have been attacked by koinobiont wasps are NOT paralysed. They continue feeding and successfully pupate. Thus, counting living larvae or pupae that have emerged from leaves may still inflate the SLM population size estimates, because these counts may include individuals that will ultimately perish during the pupal stage. Koinobiont endoparasitoids (which develop inside the body of the fly) emerge from otherwise healthy looking leafminer pupae. Thus, pupae must be retained in rearing vessels for several weeks to confirm activity of these wasps.

In summary: There are no signs of koinobiont parasitism until emergence of adults from otherwise healthy looking fly pupae.

*some idiobionts are endoparasitoids, which pupate inside the dead fly larva, and thus may be less visible within the mine, however this is a minority of species

Fig 5. Parasitoid lifecycles



A) An idiobiont ectoparasitoid lifecycle and signs of idiobiont parasitism under a microscope or hand lens, including B) a wasp larva (solid arrow) near a leafminer larva carcass (dotted arrow); and C) a wasp pupa inside a leaf mine (solid arrow). D) A koinobiont endoparasitoid lifecycle. E) There are no signs of koinobiont parasitism before emergence of adult flies and wasps from the fly puparium.

References

"Management of leafmining flies in vegetable and nursery crops in Australia" guide and "Preparedness for the exotic vegetable leafminer in vegetable and nursery crops in Australia" guide: https://ausveg.com.au/biosecurity-agrichemical/biosecurity/mt16004/#extension_package

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Images and illustrations by Elia Pirtle unless otherwise specified

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Monitoring for serpentine leafminer in Australia

Resource prepared as part of Hort Innovation project MT20005; May 2021 Version

About the pest

The serpentine leafminer (SLM; Liriomyza huidobrensis), is a well known global pest of vegetable and ornamental crops. It was first detected in Australia in October 2020, and is presently found in New South Wales and Queensland.

Actual size: 1.5-2.5 mm

Overseas experiences tell us that monitoring is a cornerstone of a successful IPM approach to managing SLM. Monitoring goals may include:

- detecting early infestations, particularly in young crops or crops for which leaf mine damage reduces marketability, such as ornamentals, lettuce and celery;
- estimating population density in larger infestations, in order to apply economic thresholds to chemical applications and to monitor the success of interventions.

This guide summarizes monitoring techniques that support each of these goals*. Each technique has benefits (✓) and drawbacks (–) and can be used in combination to effectively monitor SLM populations in Australia.

Detecting early infestations

1. Focus monitoring using predictive tools:

Monitoring is most important when crops are at vulnerable stages, or when climatic conditions make outbreak risk highest. The following tools can help identify high risk periods to guide timing of monitoring:

- The SLM seasonal activity predictor: This tool (currently in prototype stage) shows estimated activity potential for SLM across region and season within Australia. https://cesaraustralia.shinyapps.io/SLMseasonalactivity/
- The DARABUG2 lifecycle duration predictor: This tool (Fig. 1) predicts life stage durations of SLM across region and season within Australia. https://cesaraustralia.shinyapps.io/darabug2/

DARABUG2 life-cycle predictor

SLM seasonal activity predictor

2. Conduct a visual survey:

Look for signs of leafmining damage (Fig. 2) in crops to detect infestations early. For instructions, see the Hort Innovation "Preparedness for the exotic vegetable leafminer in vegetable and nursery crops in Australia" guide, which is also relevent for SLM. If transplanting plants, carefully check them both before and after transplanting. Plants that show no signs of SLM could contain eggs, which are invisible and take several days before hatching (use the DARABUG2 tool to predict the length of the egg phase).

3. Monitor for adults via sticky traps

Commercially available yellow sticky traps attract adult SLM and are especially valuable for population monitoring in closed cropping, or around young crops to indicate when adult flies may be moving into the crop (potentially from a sequential crop nearby). However, catches relate poorly to crop damage, population sizes and parasitoid activity. Traps should be hung at about plant canopy height and checked within one week.

✓ useful for a variety of invertebrate species

– difficult to relate to population size or crop damage

– underestimates parasitoid activity

In summary: Ideal for monitoring for early infestations and flights within and between farms, but poor for determining population densities

* For information on differentiating between SLM and morphologically indistinguishable non-pest Liriomyza species present in Australia, including the cabbage leafminer (Liriomyza brassicae) and the beet leafminer (Liriomyza chenopodii), see Appendix 10 (SLM Contingency Plan) of Hort Innovation Project MT16004 Final Report.

Estimating population density of larger infestations:

Possible sampling techniques include:

- Counting infested leaves with signs of damage (incl. leaf mining and stippling on leaves)
- Counting live larvae within leaves
- Counting puparia caught in pupal trays or emerged from leaf collections
- Counting adults on yellow sticky traps or emerged from pupae collections

Counting infested leaves

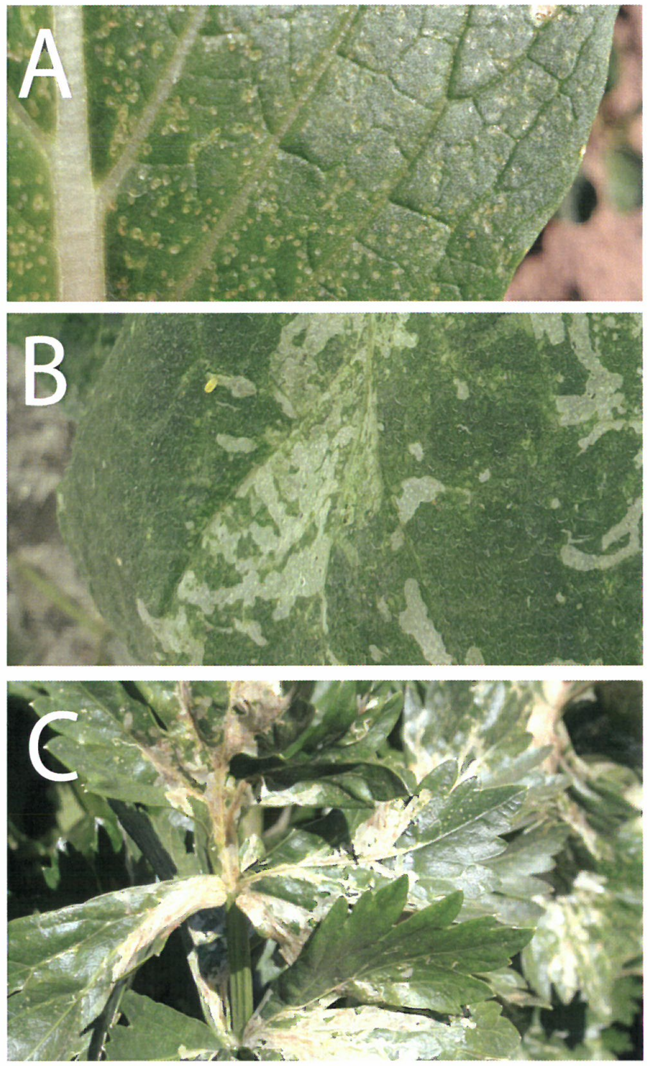
The number of leaves bearing leaf mines in a subset of leaves on a subset of randomly selected plants may be counted. However, the presence of leaf mines is not always enough to determine if intervention is warranted. Leaf mines may be old, or may contain more parasitoids than fly larvae (Fig. 3). In both cases, insecticide application would be inappropriate.

✓ easy to see leaf mines and stippling damage (Fig. 2)

– can overestimate population activity and encourage inappropriate interventions

In summary: Preferred when the goal is to detect early infestations, but may be innappropriate when monitoring the extent of an infestation or the success of an intervention

Figure 2. Leafminer damage symptoms



Damage caused by adult leafminer. A) SLM stippling damage to choy sum. Photo by: Shannon Mulholland, NSW DPI; B) SLM damage to cucumber. Photo by: Shannon Mulholland, NSW DPI; C) SLM damage to celery. Photo by: John Duff, DAFF

Counting live larvae

The number of 'active' mines in a subset of leaves on a subset of randomly selected plants can be counted by checking mines for live larvae (Fig. 3a/b) using a hand lens.

✓ accurate measure of population density

✓ accounts for idiobiont ectoparasitoid activity*

– requires a hand lens and close inspection of leaves

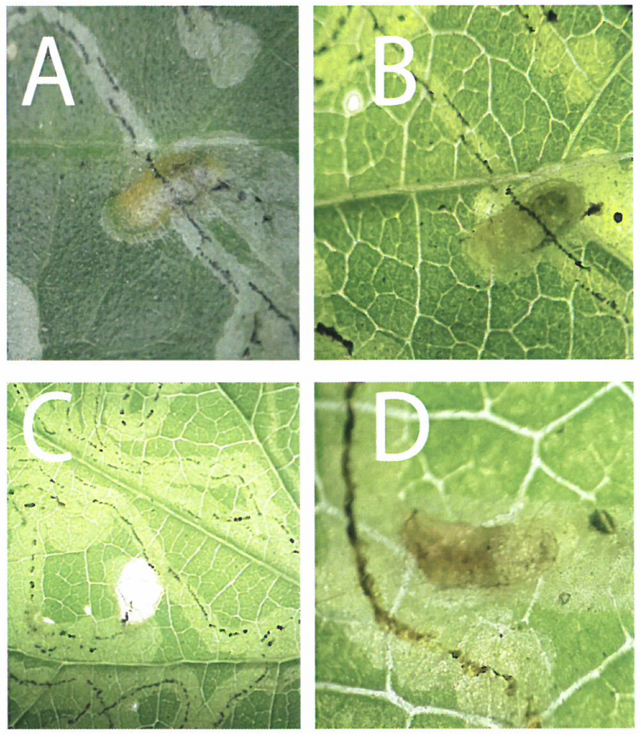
– underestimates koinobiont endoparasitoid activity*

In summary: Gives an accurate population size estimate and is a key component of sampling plans aimed at using economic thresholds (ETs)

Overseas example

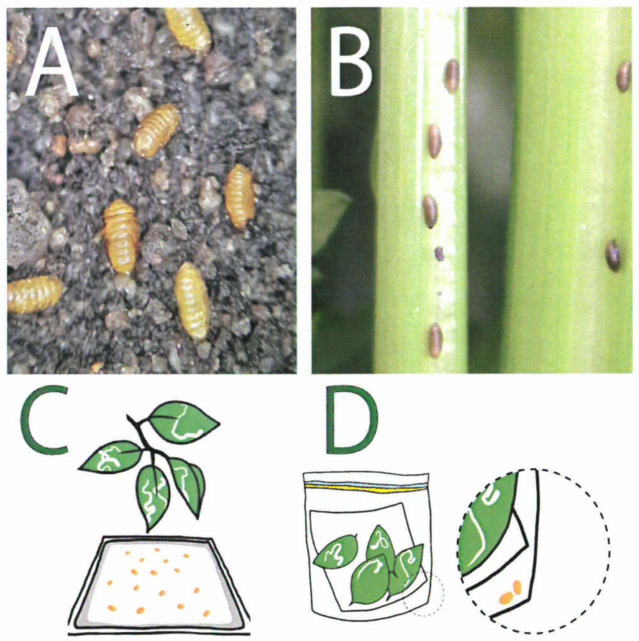
Heinz and Chaney (1995) developed the following plan for SLM in celery in California: Count either mines or active mines in 1 randomly selected petiole per plant from randomly selected plants until a density determined stopline is reached. If the population density is greater than an average 17 live larvae or 19 mines per 100 petioles, no more than 100 petioles will be required

Figure 3. Spotting larvae inside mines



A) Live larvae (VLM pictured but larvae are indistinguishable from SLM) can be seen feeding via a hand lens; B) holding the leaf up to the sun can increase visibility; C) inactive mines may be empty; or D) may contain a dead larva

Figure 4. Counting pupae in pupal trays or plastic bags of leaf collections



A) Small orange pupae (~2mm; VLM pictured but pupae are indistinguishable from SLM) accumulate in soil beneath infested plants. B) SLM pupae may collect on plant surfaces. Photo by: John Duff, DAFF. Pupae collect into C) pupal trays placed underneath plants; or D) into the bottom of plastic bags of leaf collections

Counting pupae via pupal trays or leaf collections

Leafminer activity can be measured by collecting mature larvae which have fallen into polystyrene or plastic trays (pupal trays, between 8 x 11 to 12 x 15 inches in size) and pupated over a period of 3-4 days (Fig. 4b). See the Hort Innovation "Management of leafmining flies in vegetable and nursery crops in Australia" guide for more information on their use.

Pupae may also be counted by collecting a subset of leaves from a subset of randomly selected plants into plastic bags, and observing the number of pupae that emerge and collect into the bottom of the bag.

✓ accounts for idiobiont ectoparasitoid activity*

✓ does not require a hand lens as pupae are easier to observe and count after emergence

– underestimates koinobiont endoparasitoid activity*

In summary: Popular method overseas due to being an easily visual indicator of whether leaf mine damage is caused by an active infestation, or whether the damage is old and thus intervention may be unwarranted; gives accurate population size estimates and can be used with ETs

NOTE: The pupae collected via pupal trays or via leaf collections may be retained in order to assess the level of parasitism by koinobiont endoparasitoids (see page 4). Pupae may be kept in a plastic bag (Fig. 4c) with a damp paper towel, out of direct sunlight, until adult flies or wasps emerge and adult flies may be counted

✓ accounts for idiobiont ectoparasitoid and koinobiont endoparasitoid activity*

– it can take multiple weeks for all adult flies to emerge (see DARABUG tool) and wasps even longer, thus not suitable for quick decisions

* see page 4 for explanation of idiobiont and koinobiont parasitoid and monitoring for them

Appendix 2.3
Testing and refining monitoring protocols

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by Elia Pirtle (Cesar Australia)

Image: Julie O'Halloran Qld DAF

TESTING AND REFINING MONITORING PROTOCOLS

In the previous milestone periods, on-the-ground testing of surveillance and monitoring advice and tools was unable to proceed as a part of the workshop series, as initially planned, due to COVID-19 forcing workshops into virtual formats. As a results, researchers pivoted to an informal interview approach to collect feedback on the practicality of the current surveillance and monitoring protocols and resources.

Of 12 growers and agronomist contacts provided by NSWDPI and QDAF researchers in the New South Wales and Queensland affected region, Cesar researchers were able to hold informal interviews with nine agronomists and growers representing the Fassifern/Lockyer and the peri-urban Sydney regions. The agronomists and growers interviewed represented a variety of crops, covering short growth period leafy crops such as lettuces, as well as longer growth period fruiting crops such as tomato, and also including glasshouse producers and organic produces. In phone conversations ranging from 20 minutes to over an hour, researchers solicited feedback on what monitoring protocols have proven most effective for each cropping system, where surveillance expectations of the researchers have not aligned with the developing on-the-ground experiences of growers, and what additional information or supporting tools are needed to continue to support effective surveillance and monitoring of leafminer pests.

RESULTS:

Pre-incursion awareness and early post-incursion decision making

About 40% of the individuals interviewed reported a low level of awareness of *Liriomyza* pests before the incursion of SLM into Queensland and New South Wales, while the rest either reported no awareness or did not mention their level of awareness. All individuals who did report having pre-existing awareness of the pest also reported still feeling a sense of surprise at the scale of the impact which was felt in the first year post -incursion, despite having some awareness of the pest as a potential risk. When asked about the most valuable sources of information early in the incursion, individuals most often mentioned the early series of webinars and workshops and their contact with John Duff as most valuable in the QLD region, and IPM consultants mentioned as another valuable source of early information in the NSW Region.

Early decisions tended to be informed by knowledge of similar pests, such as spinach leafminer and potato moth, both generally treated with group 28 chemicals, or starting with the list of registered chemical options, but all individuals reported a period of cycling through all available options with little success, but slowly narrowing in the most effective choices, in the first season post incursion. A valuable source of information frequently reported was other growers affected by the pest in their near area, as they traded tips about what chemicals seemed to show some control success, as well as the workshop run as part of the Lockyer Valley growers expo for those in the QLD region. When the pest slowed down its activity over the first winter post-incursion, one individual reported using the time to learn more about the pest in preparation for the next season. One individual reported making changes to their chemical plan immediately to favour softer chemistries as a result of awareness of the sensitivities of the major predators of leafminer, which two others reported starting with harsher chemistries but switching to a softer approach within their first season post incursion as a result of learning more about IPM approaches used elsewhere in the world to manage the pest. One of the individuals who had shifted to soft chemistries within the first season of the incursion reported several of his family members and neighbours also shifting to a soft approach within the second year post incursion.

Ongoing monitoring of leafminer flies

Every individual interviewed reported regular visual surveys as their primary monitoring method (with weekly and sometimes twice weekly visual inspections of crops). This did not represent an increase in the frequency of visual surveillance in response to the SLM incursion, but rather the usual frequency. Thus, the frequency of monitoring which already takes place within these horticultural systems is proving well suited to SLM management, as all interviewed individuals reported being confident in their ability to detect stippling damage early before it progressed into heavy larval feeding damage.

Half of individuals interviewed mentioned that there was a larger impact of adult feeding and egg laying damage ("stippling" damage) than they expected based on early awareness materials (and the expectations of project researchers based on experience with VLM in community gardens in the Torres Strait). Thus, individuals interviewed identified a need for a stronger emphasis on monitoring for the presence of adult flies and stippling damage (rather than focusing primarily on the larval feeding trails). About 60% of individuals who mentioned what they tended to see as the first sign of leafminer activity noted that they saw stippling damage first, and only later the larval feeding trails, and thus they considered stippling damage to be the major target of their visual surveillance. Only two individuals reported the larval feeding damage being their main target for visual surveillance. There was also relatively frequent mention of noticing the adult flies themselves on the crops as an early sign of an infestation. Three of the interviewed individuals, when asked about their ability to distinguish fresh from old signs of damage, expressed no concerns in distinguishing, and reiterated that the fresh mining was frequently accompanied by clear signs of adult activity. One individual noted that in baby leaf spinach, he tended to notice that you could roughly determine how old damage was by the age of the leaves that were affected.

About 40% of individuals mentioned relying on sticky traps as an informal monitoring technique which can assist in noticing some adult activity which leads them to check more closely for signs of stippling damage, while two individuals reported finding the sticky traps relatively ineffective and too subjective for anything other than presence/absence. One individual noted that he might only leave a trap out for an hour to tap a few individuals in order to get a closer look and confirm if the flies are serpentine leafminer, rather than trying to determine numbers. One individual reported trying vacuum sampling which was found to be too labour intensive, though sweeping was better suited to getting an informal look at numbers of adult flies and beneficial wasps. Pupal trays have not proven particularly useful for any interviewed individuals, and were reported to perform particularly poorly with low to the ground leafy vegetables, and during wet conditions.

While adult SLM cannot be distinguished from other non-pest species by eye with certainty, none of the interviewed individuals reported difficulty in determining if damage is caused by SLM as opposed to existing non pest species, simply due to SLM causing a significantly higher density of damage in its preferred hosts, in particular lettuce, kale, celery, and silver beet.

One individual reported that the speed of access to high quality montioring information for leafminer resulted in a smoother process of helping growers to learn to identify the pest than many examples in the past.

Two individuals reported that no more help is needed in monitoring, and all questions are within the space of how to control the pest. One mentioned being keen for more high-definition photos particularly of stippling damage, to increase his confidence that he is identifying damage correctly.

TAKEAWAY RECOMMENDATIONS:

- Project team to collect high resolution imagery in particular of stippling damage but also leaf mining damage on a wider range of host crops, to feature in revised extension materials.

Monitoring for beneficials

Interviewed individuals all reported some degree of difficultly in monitoring for the activity of beneficial wasps via signs of parasitism. Only one agronomist noted that they look for signs of blackened larvae at the ends of feeding trails, and occasionally attempt to rear out wasps from mined leaf samples. Two noted looking for wasps in vacuum samples, and expressed interest in learning more about what species they might be able to identify and should look for in these samples, though also noted that the commercial reality at this stage did not require specific wasps be identified. Nearly all interviewed individuals reported difficulty in relying on parasitism in the 'zero damage tolerance' leafy crops, making monitoring for beneficials a less valuable use of time. However, over half of interviewed individuals still expressed a strong interest in having information that beneficial wasps are present and active in their general region, as well as having better information about how to proactively make wasp-friendly chemical choices, regardless of whether they are able to monitor for signs of parasitism.

TAKEAWAY RECOMMENDATIONS:

- Project team to collect high resolution imagery of parasitoids being collected in affected areas to feature in revised extension materials.

Intervention decisions and chemical selection

Most individuals interviewed grow high value, zero-pest tolerance, short growing period crops such as baby leaf, spinach, lettuces and brassicas, where they reported crops might only be in the ground for 6 to 12 weeks and even minor damage can cause produce rejection. Five growers reported making the decision to intervene based on the first signs of stippling damage (noting that edge plant stippling was an early sign) paired with sings of adult activity, in order to avoid a rapid progression of damage. However, one individual reported that early stippling damage did not always progress to further damage. For example, in Chinese cabbage, they tended to see damage remain only in the older outer leaves, which reduced the impact of the damage on crop marketability. This individual expressed an interest in being able to rely upon 'triggers to intervene' tools in the future. Individuals also expressed more urgent interest in decision making support around which chemicals to use, rather than when to intervene. For example, one grower reported that the addition of serpentine leafminer to the region's pests did not cause changes in spray frequency, however has caused significant changes in which chemicals are selected for use.

Only two individuals reported feeling like they had identified a suitable chemical rotation, one being a glasshouse grower who had not seen any new activity of serpentine leafminer in the most recent growing season. However, half of interviewed individuals reported difficulty in knowing which chemicals to select (both based on efficacy against serpentine leafminer, as well as other pre-existing difficult to control pests in the system such as green vegetable bug, and on toxicity to beneficials) and reported they did not feel they had found a suitable long term chemical rotation plan yet. Individuals enquired about the possibility of a few unregistered options being tested against serpentine leafminer, including Avator Ebo, and Azamax, and Neem and Sesame oil.

When asked what tools or knowledge the respondents wished they had to optimise monitoring for and management of serpentine leafminer, the following ideas were presented:

- Ensuring high quality Australian images appear on google images
- Attractants for sticky traps
- Very simple lifecycle predictor tool based on temperature tolerances (in similar style to an existing diamond back moth predictor tool which uses a “green, yellow, orange, red” category system and also gives the predicted number of lifecycle days)
- Coordinate regular updates for an area wide chemical rotation strategy (again similar to what is used for diamondback moth)

The following preferred dissemination methods were noted:

- Webinars
- Project team could focus on disseminating information to local grower associations
- Weekly/bi-weekly text or e-mail update service (possibility of including with the DAFF beat sheet?)
- Online calculators (however these are much less preferred than an update service, with two individuals noting they very rarely go online looking for tools even though they know many exist for other pests)

TAKEAWAY RECOMMENDATIONS:

- Create a one/two page table that includes all current up to date information regarding: chemicals currently registered for use, efficacy against serpentine leafminer (including formally measured international data as well as, where possible, informal Australian anecdotes for what chemicals are providing the best control), and toxicity to beneficial parasitoids (including formally measured international data where possible for cosmopolitan wasp species, and making educated assumptions for poorly studied Australian wasp species)
- Create an online lifecycle predictor tool which gives a “high, medium, low” risk rating for activity (based on the establishment model predictions), as well as the current prediction of lifecycle length (already available as the DARABUG tool), and the option to sign up to a weekly e-mail service which reports activity risk and lifecycle length
- Identify all grower networks in affected areas which have individuals who are key sources of information in the community, who can be the focus of extension efforts to share tools and predictions, or identify already existing information sharing services (such as Qld beat sheet) into which regular serpentine leafminer prediction updates could be built

IN SUMMARY

Overall, interviewed individuals expressed satisfaction with the current surveillance and monitoring advice and resources, aside from a request for additional high resolution photos, particularly around stippling damage. All interviewed individuals expressed a heavy interest for the project’s focus to move ahead towards building appropriate chemical management plans that would be soft on wasp populations but still provide effective control of serpentine leafminer.

Interview Questions:

What had you heard about *Liriomyza* pests before seeing them on your properties?

QLD 3	On our radar, caught a bit out, there was a big rainfall event make it hard to access the spot and it was isolated spot, looked almost like spray residue at first, then looked closer and found the actual mines, still not too sure or worried (was iceberg), and was about 2 months ago also in other crops celery and baby leaf
QLD 5	Aware of it through grower meetings, never expected so much damage! Couldn't let it take its course
QLD 8	Definitely slowed down this year, didn't flare in winter, and in summer periods only about ⅓ of prev seasons at worst (strategies have definitely changed) Seeing more parasitism Still doing some trapping and rearing etc but just so much time involved, and now John has facilities and he an just send over
QLD 9	Around Nov fist sign Have been growing beans, what they were found in first time He looks for actual mines, sometimes finds similar looking adult insects but sent a sample a sample to John recently but no damage seen so might be different miner Used some abamectin for initial infestation, then went to low chem approach beyond that, no group ½, but did have green veggie bug flare up that needed some extra chemical control
NSW 2	First time he'd heard of it when it arrived
NSW 1	zilch

NSW 3	He got called in by Chris because Andy know the local Elders guy who does agronomy and chem sales, was the worst he'd seen it before even compared to 'ground zero' in hydroponic lettuce Surprising but also 'expected' in a way He has been trying to push the elders guy into IPM and gives him advice, Chris was using reasonable chems but just overuse…had to work out the new program on the ground for both adult and larval control He relied on his experience as an IPM consultant, he's an applied entomologist so already quite familiar with leafminer Early webinars were really helpful Surprise to see how much physical damage by adults! Lots of good helpful work in the biosecurity projects helps us get up quicker, but needing to work out the program for both adults and larvae a big challenge And then bring wasps in, and just how 'soft' are soft chems for the wasps particularly? While there is info of chemicals effect on wasps its still confusing and hard to find, and he thinks this is the next step - advising specifically chemicals safe on our wasps that can be adult knock down. Chris works with glasshouse growers too, and so far so good in the glasshouses…
QLD 7	17 months so far in this agro job - hearing from John and also from Biran Thistleton got his started, he didn't have much to do with it last year but this year popping up more ex in a tomato block Esp silverbeet and chinese cabbage, some in tomato And has been pushing the soft chem story quickly - granite belt

How did you decide what to do when you first found *Liriomyza* pests?

QLD 3	Looked at some options, he knew a bit about spinach leafminer, and they tend to go to group 28s for chewing insects at that time anyway, so just make sure fist spay at first true leaves and it usually worked for spinach leafminer, so went that way first, but numbers exploded anyway, they rotated through all, and things settled with the weather cooling, then learned about the pest all winter

NSW 2	Chatting to other growers
QLD 5	Start with all registered options of chemicals, then narrowed in once ID'd which were most effective and 'stumbled through"
QLD 4	Appeared in downs first so they got a heads up, and then John ran workshops Got ID info and good info, before they ever saw it In a neighbour's tomatoes and then headed over into baby leaf and John warned right away to avoid heavy chemistry, so they used the yellow sticky traps right above crop and right away got flies, so next thing to address mines on leaves went to look for soft Chem and growers not too keen on that so had to push for it, ended up using coragen, and within three plantings back on top of it, lost about 50 out of 200 Meters of rows "Yellow fruition traps" He does lots of potatoes on farms, use group 28s for potato moth, and if any miner there it seems effective there to. Less concerned with very first flight in, just keeping an eye on how numbers build
NSW 1	Started noticing cause his cousin 4 or 5 ks up the road saw it first and had major problems flaring, then he saw some in the spinach and silverbeet and then within 4 months become big problem (literally though they'd have to change crops!). He is all leafy veg, kale silver beat lettuce (kale seems not a problem). They had quite a few spring and even summer issues even though 30C (oct to about March), but it was also around in winter A tomato grower across the road not having such problems Chain stores can be really picky… No one seemed to have much knowledge, whoever you spoke to, so he did lots of his own research and found lots of mentions of everyone spraying hard right at the start but that causing more problems and shift to IPM So he stopped using the knock down and shifted to targeted sprays, been about exactly 12 months since no more knock downs, previously using a 'soft' knock down' and used for about 4 months - then shifted to targeted sprays, and took a little while for effect to kick in and now haven't seen much until today actually. He's trying to use a couple chem in rotation (success mostly for adults and transform for the larvae), his brother in law in hydroponics doing the same now (was spraying three times a week and couldn't get rid and after 6 months finally stopped using it)

	Lots of folks went in hard with knockdowns and took soft approach a bit later, like his neighbour, and neighbours dad now…but most people seem on it now…
QLD 7	Everyone used to spraying every week (lannate but it knock out predators but learned that one was hard on predators) still most people not on soft approach

How are you monitoring for *Liriomyza* pests?

QLD 3	Physical inspections have been the big one, just another thing to watch for when also looking at crop health nutrition etc…they often see stippling more than mines He feels he can look like mines/stippling and they look different from spinach leaf mine vs serpentine… His question - one you have a huge pop, all the flies look the same, but he things he can tell spinach from serpentine by a 'vibe' of colour and behaviour Once you can fly the flies, so looking for adults, then a problem, particularly starting from edges
QLD 5	Physically in the crop every week, and esp when weather conditions correct, they are using stickies too Stippling has been most useful to look for, seems to be showing lower in crop (celery worst hit, followed by shallots/onions, lettuce, all cabbages, baby leaf) Sticky traps - just used infrequently because when physically there you see it soon enough, adult movement is what traps are best for, so put them op when you've got early crop stage of a susceptible crop
QLD 8	They religiously check twice a week, its a bit extreme, and first check is more in depth than second Establishment costs are so high 20,000 hectare, and getting 50,000 out of it Some crops have zero tolerance because of the market

	Monitoring is just not trick now its the control
	Mostly visual surveys, mostly looking for the twirls Tomatoes and cucumber This season much better than before, big reduction this year Found good chemical control in glasshouses - success is the name Sticky traps felt like waste of time - much more time effective to look for visual damage No beneficial in glass glasshouse He's curious about potentially predicts effect of rain?? His suppliers for chemicals they are first stop for info
QLD 4	sees stippling first, ex in his baby leaf, sent samples over to John, but got no larvae found inside
NSW 1	Was using some sticky traps, Andy R was coming out to the farm every couple weeks and set traps to see what's around (heliothis too) and they seemed useful for noticing if adults are around When harvesting, always looking at what's around (mines or flies) Quick walkthroughs if time Sees both stipple and mines - today was first time he saw the mines but no stipples… And stippling is enough to make the leaf too ugly…last year so bad the bottom leaves actually drying up

NSW 3	Monitoring first phase is pretty excellent, has helped growers a lot more than some examples in the past Not really any issues in identifying pest now, and growers are very interested in beneficials story but need for chemical info - but without getting new info but just stronger with the ‘logical guess’ And stress the damage by adults in future extension materials! Its been bigger issue that expected!
QLD 7	Saw some leafminer tracks (grower picked it up first in wet area near paddock in blackberry nightshade then suddenly in crop), probably 40% leaves marked in the creek area, and grower kept using lannate, then switch to abemectin for heliothis and leafminer and ‘belt’ group 28

Have you found any tools or resources particularly useful?

QLD 3	In cotton he used sweeps and vacuum, so he tried that here, just to look at beneficial etc and numbers of adult flies Vacuum is a bit too labour intensive but does paint a picture, he doesn’t feel like much faith in yellow stickies, they are too subjective (it depends where you put them, five meters apart such a large difference in numbers you get, they seem good for presence absence) He uses small car vac with a pantyhose haha
QLD 5	Not really, monitoring is not where help is needed, its intervention
QLD 8	Stickies have been used to just figure out what all the small insects that are flying round actually are - they don’t even need to put them on long, even just for an hour, They don’t always act on seeing something on the sticky trap But the trays not so commonly used, and less valuable because he knows that they are looking for and the adults and stippling and also pupae stuck on plants very visible when numbers get very large

	And so much rain this season, made the traps very tricky fill with water
QLD 7	No traps, grower needs to pull out the nightshade weeds,

How are you making your decisions on whether intervention is necessary?

QLD 3	Lots of it comes down to the adult activity in combination with early stippling counts Low to moderate adult numbers tend to see edge stippling and then progresses forward…
QLD 5	All crops are high value zero tolerance, so the first sign is the trigger Life cycles of crops are quite short, maybe 6 to 8 weeks, maybe brassicas as long as 12 Issues more around intervention than monitoring, monitoring is not where help is needed, its intervention
QLD 8	Would like to be able to rely on 'triggers to intervene' tools into the future They know some cops get attached like chinese baggage for ex. But only attacks the outside leaves so haven't changed practice much, just using knock downs, and only got the outbreak in the celery next to the cabbage but never really cabbage - not sure if because the pop just died out or they just don't like it enough as the plant ages… Some farmers still do have some difficulty identifying or even acknowledging/believing in the area… Not great attendance to the first workshops

	They have three chems they thinking are working well and they don't use until monitoring sas they need to First thing they spot is adults, while you can't tell exact species, and the stippling which usually starts first Mines are there too but stippling/adults is a better first indicator which doesn't always turn into something So when do you decide it does need intervention? That's more about damage to leaves and more mining being present
QLD 4	checks crops weekly, so can assess and react very quickly, he doesn't really have many rule of thumbs for other pests to help decide when to intervene, To get that trust in beneficial, early 2000s dBm huge problem, probably 50 of the 200 growers in the valley still use heavy chemistry so now and again DBM still gets out of hand and still have some agros leading use of heavies even though feel like we already learned that strategy doesn't work well bc DBM, so felt familiar BC DBM story already
NSW 2	not feeling like to much need now, he feels its manageable enough in the glasshouse because he found the right chemical, rule of thumbs could work but he feels they tried a lot to give lots of info but end of the day it always comes back to hearing what worked for others. It was really bad last year so everyone asking each other what happened. Every day he is in the crop!
QLD 7	Mostly the spray frequency not too changed but just changing the choices Ex. got veggie bug recently, not too many options - so if we need to keep things soft then we need an option that works for veggie bug so he's trying to confirm if avatar eVo will do green veggie bug - still unclear Grower meeting 4th of May he will invite John and need to be able to make chemical recommendations Used to work for an organic chem company - azamac sesame oil they are confident can be registered soon… In the tomatoes, also can control tomato leafminer with abemectin, and they move into fruit In leafy things, he says talking to that grower they were in outer leaves recently

Are you confident in distinguishing old damage from active damage?

QLD 3	He doesn’t look for the larvae specifically, he sometimes takes leaves home for his digital microscope He reckons you can almost tell in baby leaf crop based on position of leaf determining age of leaf… not as good for iceberg and some other Feels okay distinguishing very old vs very fresh (based on necrosis) He hadn’t heard of frass, might try that to help ID fresh
QLD 4	Never really a challenge, old looks quite different than fresh, and paired with the stickers is the best cue for if adults are flying around, and if the grower sees the fly in the trap it's a good convincing visual Not sure if he's always IDing exact species But less important since they aren't zero tolerance For tomatoes shipping interstate they do have to spray heavy insecticide to eliminate fruit fly before harvest - really needs legislative review
NSW 1	Straightforward to tell what it is, won’t get confused He thinks even there is all these other non pest native flies, surely these pests could be well controlled too eventually

Have you ever identified signs of parasitism in an active *Liriomyza* population?

QLD 3	He had definitely seen hemiptarsenus, with the clear antlers it helps Commercial reality probably doesn’t need to identify specific ones at this stage Just work on softer chemistries where possible

NSW 1	He’s asked Andy (entomology background) to get on board for looking for beneficials Andy uses vac sampler he thinks works well
QLD 8	Very keen to know what beneficial species to be looking for 1000L water contains have been converted to big flower pot type things to have flowering plants to attract beneficials, they are also working with bugs for bugs sending samples, cause cant buy large enough batches of beneficials But don’t really know what to put in them for leafminer… Would love to have more info/ideas about how to leverage non pest plants/weeds to move into crops He doesn’t have any crops that have that sit and wait period before the fruit is affected… Would be great to be able to see what parasitism looks like but again its pretty close to zero tolerance…
NSW 3	He looks for black larvae or pupae as a sign of parasitism and checking weeds is a key thing He does vac sampling as a standard - same with yellow stickies but not great in the field esp with rain or watering Hasn’t tried pupa trays and sees it could be good in a polytunnel but not great in field

What tools or knowledge do you wish you had to optimize monitoring for *Liriomyza*?

QLD 3	Good info on lifecycle time and its dependence on temperature Need something pretty simple - ex diamondback moth they have ‘green yellow orange red’ category system -

	They also use lifecycle days, they get sent weekly text message with the average lifecycle that week in lifecycle days - that comes from Zara's group, also had some notes about where you're at in rotation He thinks it was all in same text message…(or maybe email for traffic light) And then have another link for more technical details for the 'nerdy' folks like him
QLD 9	Went to John's seminar and that was great looking at the specimens, then looking early in spring and late in the autumn which are the vulnerable stages, particularly looking in spring but didn't show up He hasn't replied much on external tools, but anything that helps with visual observation, he goes straight to google images
QLD 5	Not much valuable info available that helps build the chemical plan Some of it is the first step of what chemicals would work (they feel like he's got a good sense of what's good for beneficials from experience with other pests) Working out what chemicals do work is still on ongoing exercise…still requiring experimentation
QLD 8	Online calculators, lots of people looking for them early on, ex. DBM has lots of timing calculator tools that seem quite accurate, also thing about DBM is there's specific strategies t use so the texts also have the updates for the chem strategy etc Lockyer valley growers group really good at texting and emailing info around There are so many tools but he rarely goes online to them… Once of week email in QLD by DAFF beat sheet and its really good for staying across all pests He didn't find such a little range of temp tolerances, ex they didn't find them dying out at 30C and above for example. For example many days of 40C last season and not much change in pop

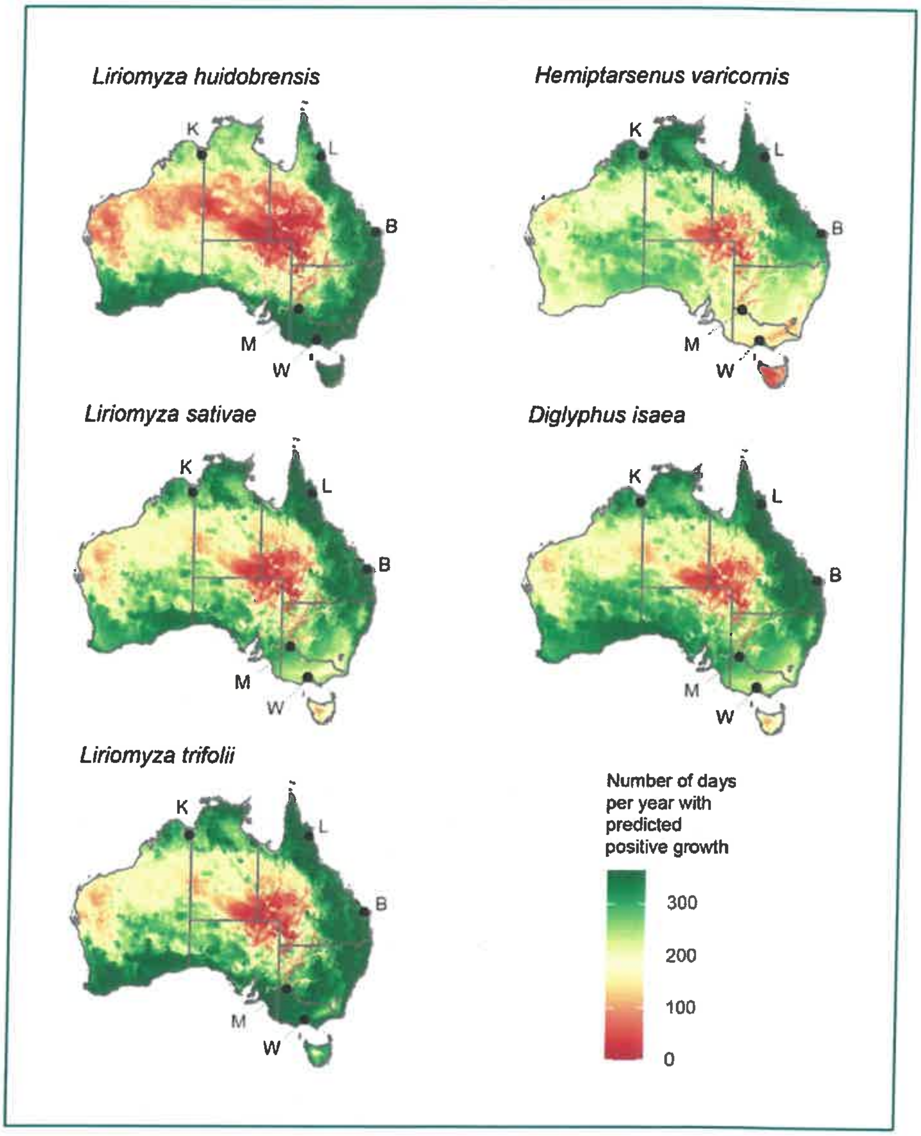
QLD 4	Greg: difficult question, ever week we have to monitor, need numbers and pressure and make decisions week to week, if he sees a dBm egg that's a worry he tries never to let them hatch, An attractant for the traps would be very high value, both for monitoring but also to bring pop numbers down, just like fruit fly and cucumber fly All biological info is good to have Doesn't go online much to get online tools, he went to all John's presentations Some agros are still struggling even with DBM, ex the link to temperature
NSW 1	Would like to see more options still for better rotation He's never had a tool like described of the DBM - he's got an agro from the chem company who gives update if somethings going around As far as monitoring - within Sydney basin he reckons most people pretty aware esp the advisors and chem sales guys - but outside Sydney basin One there thing to add - the temperature things, we had a slow summer with lowish temps but then leafminer just dropped dramatic with that uptick in summer temp - so this is good messaging to promote better For temp assistance monitor tools - horticulture growers aren't so used to 'predicted info' compared to broadacre, I think the reason these tools aren't used so much in horticulture is just technical skill level to appreciate these tools. Some of his veggie growers only just getting good at e-mails No one playing that role so much in the peri urban Sydney growing area - its an extremely diverse group of growers ethnically and technically in that area, and a bit more 'outside suspicion' than places like Lockyer and larger hort areas, bit of a thankless task for local land services and they can only penetrate so deeply…he does love going to the few growers associations that have cropped up ex Cambodian growers assoc Again he thought the webinars were a great format

Any final thoughts

QLD 3	End of the day about being in paddocks and looking for subtle signs early enough He would really love to see more high def photos just to help him feel more sure They've had a massive rainfall event all summer - way greener than usual - maybe more diluted pests this year?
QLD 5	Not really sure what he needs yet, still in learning phase…
QLD 8	They even get veggies went back with spiders as a class offense, same level as glass!!! Would love to see chemical options ex azamax neem products generally regarded IPM friendly They found best thing working is geographical separation, keep crops separated on separate farms, and rotate between farms, Its like DBM, there's no getting away from chemicals of some sort, so need a clear plan of what works
QLD 4	Nothing better than revision, so good to keep the monitoring story,
NSW 2	It's all pretty good

Appendix 2.4

Forecasting the potential distribution of invasive leafminer pests, *Liriomyza* spp. (Diptera: Agromyzidae), and their natural enemies



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ORIGINAL ARTICLE

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Forecasting the potential distribution of invasive leafminer pests, *Liriomyza* spp. (Diptera: Agromyzidae), and their natural enemies

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Abstract

Three species of polyphagous *Liriomyza* leafminers (Diptera: Agromyzidae), *Liriomyza huidobrensis*, *L. sativae*, and *L. trifolii*, are internationally significant pests of vegetable and nursery crops that have each been recently detected on the Australian mainland. Due to the early stages of these invasions in Australia, it is unclear how climatic conditions are likely to support and potentially restrict the distribution of these species as they expand into novel ranges and threaten agricultural production regions. Additionally, it is unclear how natural enemies, particularly parasitoid wasps, will mitigate the impacts of these pests. Here, we predicted the future establishment potential of *L. huidobrensis*, *L. sativae* and *L. trifolii* in Australia, as well as two cosmopolitan parasitoid wasps known to provide control of the flies in both field and glasshouse settings, *Diglyphus isaea* (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae). Global distribution data spanning 42 countries were compiled and used to validate a process-based model of establishment potential based on intrinsic population growth rates. The modelling approach successfully captured the international distribution of the three *Liriomyza* species based on environmental variables and predicted the high suitability of non-occupied ranges in Australia. The largely unfilled climatic niche available to these pests demonstrates the early stages of their Australian invasions and highlights locations where vegetable production regions are at particular risk. In addition to Australia, our results highlight many regions globally where *L. sativae*, *L. trifolii* and *L. huidobrensis* have the potential to spread in the future. Within Australia, *D. isaea* and *H. varicornis* are predicted to have a large spatial and seasonal overlap with each *Liriomyza* species and thus are expected to influence the future spread of these pests and play an important role in local pest management programs.

KEYWORDS
Agromyzidae, biosecurity, ecological niche, environmental envelope, mechanistic model

INTRODUCTION

The incursion and establishment of invasive alien species is an ongoing challenge around the world, which is mostly being driven by increasing global trade and the movement of humans (Hulme 2009). The invasion of some species into novel environments can result in substantial negative impacts on agricultural productivity (Pimentel et al. 2001). One such group of exotic species are the polyphagous *Liriomyza* leafminers, belonging to the family Agromyzidae. The Agromyzidae are a well-known group of small, morphologically similar flies whose larvae feed on plants, often as leaf and stem miners. The majority of agromyzid species are very host-specific, however there are some highly polyphagous species, which have become important pests of agriculture and horticulture in many parts of the world after spreading from their native ranges (Spencer 1973, 1990). These include the serpentine leafminer (*Liriomyza huidobrensis* (Blanchard)), the vegetable leafminer (*Liriomyza sativae* Blanchard), and the American serpentine leafminer (*Liriomyza trifolii* (Burgess)), all of which have historically been identified as high biosecurity risks to Australia (Jovicich 2009). In 2008, *L. sativae* was detected for the first time throughout the north Australian islands of Torres Strait (Blacket et al. 2015), and then on the Australian mainland at Seisia in 2015 (International Plant Protection Convention 2017). The pest has not yet been detected in any other regions of Australia despite ongoing surveillance efforts. Then in late 2020, *L. huidobrensis* was detected in the Sydney region and south-eastern Queensland and eradication was subsequently deemed unfeasible (International Plant Protection Convention 2021a; Mulholland et al. 2022). Early in 2021, *L. trifolii* was detected in northern Western Australia and within the Torres Strait, and final considerations on technical feasibility of eradication are still underway (International Plant Protection Convention 2021b).

The majority of crop damage caused by pest *Liriomyza* species occurs when the larvae feed between the upper and lower leaf surface, which curtails photosynthetic ability and reduces marketability of some crops (Parrella 1987). The most effective natural control of these pests comes from parasitoid wasps (Ridland et al. 2020). Mortality results from parasitism, or by stinging and host-feeding by adult wasps. However, appropriate chemical controls remain a key management tool, particularly when there are incursions into new regions due to the unfamiliarity with the pest (Parrella & Keil 1984; Rauf et al. 2000). Overseas, where these three *Liriomyza* species are established in agricultural production areas, insecticide-based control disrupts parasitoid populations as well as beneficial predators, often leading to secondary pest outbreaks (Murphy & LaSalle 1999; Parrella 1987). These global experiences support the notion that polyphagous *Liriomyza* spp. leafminer are secondary pests, typically only reaching damaging levels after severe reductions in parasitoid numbers (Parrella & Keil 1984). Reductions in parasitoid populations can occur through a number of processes, but are mostly related to insecticide usage (Parrella & Keil 1984). In fact, the application of broad-spectrum insecticides can have a greater negative impact on parasitoid populations than the *Liriomyza* spp. leafminers, which are largely protected inside the leaf during egg and larval development (de Little et al. 2020). For example, some carbamates, organochlorines, organophosphates and pyrethroids have high toxicity to parasitoids but show limited efficacy against *Liriomyza* spp. (Hara 1986; Hidrayani et al. 2005). Furthermore, insecticide resistance, which has been documented in all three *Liriomyza* species and is particularly common in *L. trifolii* (Ferguson 2004; Keil & Parrella 1990; Reitz et al. 2013), will exacerbate these issues and further increase the likelihood of local pest outbreaks.

Within Australia, there are at least 50 species of parasitoid wasps that attack native and adventive agromyzids (Ridland et al. 2020). The invasion of *L. sativae*, *L. trifolii* and *L. huidobrensis* will see them entering ecosystems that harbour established agromyzids, such as *Liriomyza brassicae* (Riley), *Liriomyza chenopodii* (Watt), *Phytomyza syngenesiae* (Hardy), and *Phytomyza plantaginis* Goreau, as well as their parasitoid wasps, consisting of a range of eulophid wasps, such as *Diglyphus isaea* (Walker), *Hemiptarsenus varicornis* (Girault), *Neochrysocharis formosa* (Walker) and *Zagrammosoma latilineatum* Ubaidillah, as well as a range of braconid wasps (*Opius* spp.) and pteromalid species such as *Trigonogastrella parasitica* Girault (Ridland et al. 2020; Xu et al. 2022). Based on overseas data, these parasitoids are expected to attack *L. sativae*, *L. trifolii* and *L. huidobrensis* where they co-occur in Australia (Schuster & Wharton 1993; Zaifu et al. 1999). *Diglyphus isaea* and *Hemiptarsenus varicornis* are both cosmopolitan species, and their value in regulating populations of *Liriomyza* spp. has long been recognised (Hondo et al. 2006). *Diglyphus isaea* is the primary species used for augmentative biocontrol of *Liriomyza* spp. in glasshouses in Europe, North America and Japan (Chow & Heinz 2004; Hondo et al. 2006; van der Linden 2004). *Hemiptarsenus varicornis* is found in Asia and Africa and is one of a number of parasitoid species regulating unsprayed *Liriomyza* populations (Ridland et al. 2020). These species are expected to form a key part of ongoing management programs in Australia, however, the seasonal activity of these parasitoids across Australia will be crucial in determining the overall efficacy of biocontrol (Ridland et al. 2020). Unfortunately, there are large knowledge gaps that exist around the current distribution and seasonal activity of *D. isaea* and *H. varicornis* within Australia. Furthermore, the distributions of the three pest *Liriomyza* species will almost certainly continue to expand and thus also remains unknown. This limits our ability to predict the likely impacts of biocontrol in the field and thus effectively prioritises monitoring and pest management efforts.

Knowledge gaps in current and future distribution of these pests and natural enemy complex can be aided by species distribution models (SDMs), which use environmental data to predict spatial patterns in the occurrence likelihood of species (Elith & Leathwick 2009; Kearney & Porter 2009). There is some limited research into estimating species distributions of these *Liriomyza* species. Kroschel et al. (2016) measured life history data on *L. sativae* at different temperatures and estimated development rates globally. However, only temperature was considered (not moisture) and mortality at extreme conditions was ignored. Relatedly, using CLIMEX software, Jovicich (Jovicich 2009) modelled the potential future geographic distribution in Australia for these three *Liriomyza* spp. (as well as for *Liriomyza bryoniae* (Kaltenbach), and *Chromatomyia horticola* (Goureau)) but provided little biological justification of the methods

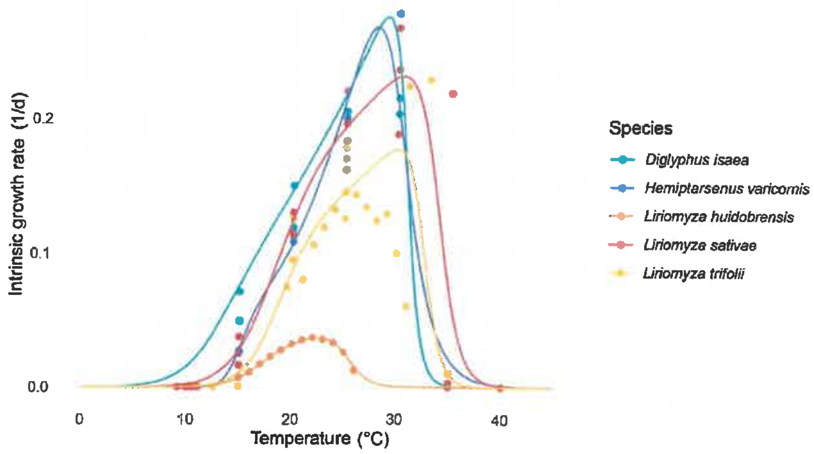

FIGURE 1 Intrinsic rate of population growth at various temperatures with parameters fitted to compiled data provided in Table S1. The responses of population growth rates were captured using a non-linear function described by Schoolfield et al. (1981) and fitted to data on *L. sativae*, *L. huidobrensis*, *L. trifolii*, *D. isaea* and *H. varicornis*.

nor validation using the known international species distribution.

Here, we incorporate biological and ecological knowledge to forecast the establishment potential of *L. sativae*, *L. huidobrensis*, and *L. trifolii* in Australia, and: (i) identify environmental drivers of the known current global distributions; (ii) identify areas in Australia of high climatic suitability; (iii) explore seasonal variation in population growth potential and; (iv) identity vegetable production areas at greatest threat to each pest as measured by climatic suitability. Then, using the same modelling approach, we assess the potential distributions of *D. isaea* and *H. varicornis* in Australia, and determine their likely overlap with *L. sativae*, *L. huidobrensis* and *L. trifolii*.

MATERIALS AND METHODS

To estimate the potential distribution of *L. huidobrensis*, *L. sativae*, and *L. trifolii*, in Australia based on population growth potential, for each species we (i) compiled empirical data on the temperature response of intrinsic population growth, (ii) projected estimated population growth across the world via modern global climatic data sets, and (iii) validated the predicted distributions using available occurrence data. For the parasitoids, *D. isaea* and *H. varicornis*, due to a lack of available occurrence data, only steps (i) and (ii) were undertaken.

Population growth potential

The intrinsic rate of population growth is the exponential growth rate of a population when growth is not limited by any density dependent factors. More formally, if the change in population size N with time t is expressed as $\frac{dN}{dt} = rN$, then r is the intrinsic population growth rate (individuals per day per individual), which can be decomposed into per capita reproduction and mortality rate. The intrinsic growth rate parameter r depends strongly on temperature, with population growth inhibited at low and high temperatures (Haghani et al. 2006), and can be described using a variety of non-linear functions. Here, on the basis that negative population growth cannot be reliably measured under common population cohort studies where reproduction must be positive (i.e., a negatively growing population cannot be maintained in the laboratory), we separated the parameterisation of the positive intrinsic growth rate r_p from negative growth r_n. The temperature response of positive growth rate (Figure 1) was modelled using a formulation of the Sharpe and DeMichele model and parameterised from empirical data (Tables 1 and S1) (Chien & Chang 2007; Haghani et al. 2006; Zhang et al. 2000) and non-linear least squares regression.

Population mortality from extreme conditions

Extreme stressor mortality can be assumed to occur once an environmental variable s exceeds some threshold (e.g., critical thermal maximum), beyond which the mortality rate scales approximately linearly with the depth of the stressor (Enriquez & Colinet 2017). Stressor-induced mortality was incorporated through quantifying the threshold s_c beyond which stress-associated mortality commences and the mortality rate parameter m_s that reflects the per capita mortality per stress unit per time (e.g., degrees beyond the stress threshold per day). The mortality rate for the ith stressor was incorporated as $\frac{dN}{dt} = (r_p - r_n)N$ where $r_n = \sum_i f(s, s_c) m_s$ and $f(s, s_c)$ is a function that provides the positive units by which s exceeds s_c. In calculating heat stress, for example, $f(T, C_{T_{max}}) = \min(T - C_{T_{max}}, 0)$ when T is temperature $C_{T_{max}}$ is the heat stress threshold.

Here, we considered the thermal stressors (heat and cold) as well as moisture stress, which were estimated following Maino et al. (2021). *Liriomyza* spp. are herbivorous species, so rather than soil moisture we used the

TABLE 1 Parameters for critical thresholds and mortality rates for key environmental stressors for *L. sativae*, *L. huidobrensis*, *L. trifolii*, *D. isaea* and *H. varicornis*. See Maino et al. (Maino et al., 2021) for estimation methods

Parameter	Description	Species: *Liriomyza sativae*	*L. huidobrensis*	*Liriomyza trifolii*	*Diglyphus isaea*	*Hemiptarsenus varicornis*
$C_{T_{min}}$	Critical minimum temperature, °C	10	−5	5	10	15
$m_{T\,min}$	Mortality rate per cold stress, °C/d	0.1	0.029	0.07	0.07	0.07
$C_{T_{max}}$	Critical maximum temperature, °C	35	30	35	33	37
$m_{T\,max}$	Mortality rate per heat stress, °C/d	0.365	0.05	0.2722	0.365	0.365
$C_{wilting}$	Critical wilting fraction, –	0.8	0.8	0.8	0.8	0.8
$m_{wilting}$	Mortality rate per desiccation stress, 1/d	11.5	2.5	7.83	2.5	2.5

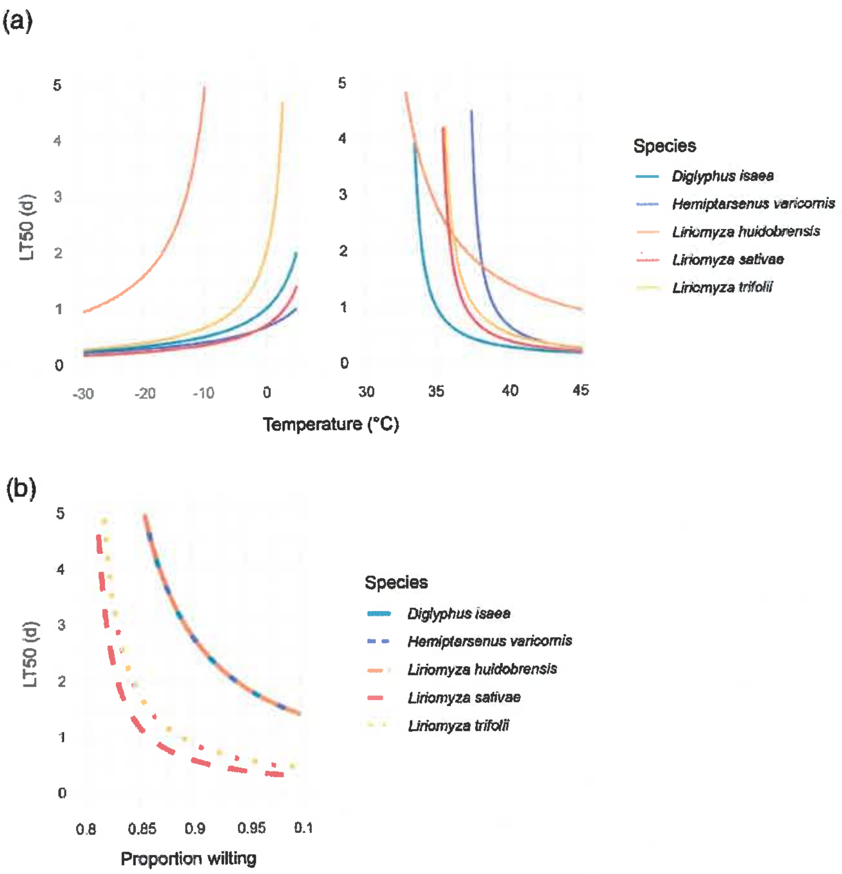

FIGURE 2 Estimated stressor mortality rates expressed as time to 50% mortality (LT_{50}) from cold and heat (a) and desiccation (b) for *L. sativae*, *L. huidobrensis*, *L. trifolii*, *D. isaea* and *H. varicornis*.

proportion of plants at wilting point to be more relevant, as it considers the effects of soil type on water potential. When these thresholds were exceeded, the mortality rate m_s for each stressor s was estimated from previous studies using the solution to the differential equation when intrinsic growth rate is negative, $p = e^{-m_s a_s t}$ where p is the surviving proportion and a_s is the accumulated stress units until time t. Table 1 provides estimates for threshold parameters for climatic stressors and

Figure 2 represents the estimated stressor mortality rates expressed as time to 50% mortality (LT_{50}) from cold and heat (Figure 2a) and desiccation (Figure 2b). Using the stressor model previously described, the equations used to compute the curves in Figure 2 for each stressor are: $min(C_{T_{min}} - T, 0)m_{T\,min}$ for the cold stressor, $\min(T - C_{T_{max}}, 0)m_{T\,max}$ for the heat stressor, and $\min(W - C_{wilting}, 0)m_{wilting}$ for the desiccation stressor, where W is the wilting fraction.

Simulating global population growth potential

The Soil Moisture Active Passive (SMAP) data products derived from the SMAP satellite mission were used to estimate various climatic conditions relevant to habitat suitability for *L. sativae*, *L. huidobrensis* and *L. trifolii* (Entekhabi et al. 2010). We used SMAP Level 4 data products, which are model-derived value-added products that combine SMAP satellite observation with a land surface model and observations-based meteorological forcing data, including precipitation and temperature to provide global gridded climatic and environmental data at the 9 km resolution every 3 h from April 2015 until present since satellite mission commencement (Reichle et al. 2017). Validation analysis has revealed that SMAP data products are generally better than other comparable data sets (Reichle et al. 2017). Three SMAP data fields were used to define climatic stressors including 'surface_temp' (the mean land surface temperature [K]) and 'land_fraction_wilting' (the fractional land area that is at wilting point based on soil moisture at 0–5 cm [m^3 m^{-3}] and soil type).

Using daily SMAP climatic data from 2017 and the parameters defined in Table 1, for each month, the mean daily stress limited growth rate $r - \sum_s f(s, s_c) m_s$ was calculated using 3-hourly timesteps. These monthly values were then used to derive summary statistics, such as mean positive monthly population growth, or the number of months with positive population growth per year. These summaries enabled us to collapse the seasonal variation in growth potential into one temporal dimension to aid visualisation. We summarised these temporal fluctuations by summing the positive growth across months to estimate maximum growth potential, as well as mean growth rate, which includes negative growth and thus represents population persistence.

Goodness-of-fit

To validate the adequacy of the model output with observation, we use the Boyce Index (Hirzel et al. 2006; Jiménez & Soberón 2020), which provides a measure of the goodness of fit of the model for the leafminers species. The model outputs the number of days in the year with positive growth rate. This output was directly compared with occurrence records for each species compiled during a literature review of global distribution data for all five species spanning 42 countries.

The Boyce Index is the Spearman rank correlation coefficient computed between the Boyce ratio F_i for month i and the number of months covered by the predicted number of days with positive growth n_i (i.e., 1 month when the number of positive days is between 0 and 31, 2 months for 31 to 59 positive days, and so on up to 12 months). The Boyce ratio is defined by: $F_i = \frac{P_i}{E_i}$ with $P_i = \frac{p_i}{\sum_{j=1}^{12} p_j}$ and $E_i = \frac{a_i}{\sum_{j=1}^{12} a_j}$ where a_i is the number of pixels with value n_i and p_i the number of data points falling into pixel with value n_i. Thus, a Boyce Index of 0 means that the model is no different from the random model (random selection of points), a positive value indicates a good predictability whereas a negative value indicates an incorrect model.

Comparison of vegetable production regions

Seasonal and regional variation in estimated population growth rates were explored for five major vegetable production regions in Australia. These were chosen based on total production volume of affected agricultural commodities and variation in climatic conditions (ABS 2017). This included Lakeland (Queensland), Bundaberg (Queensland), Kununurra (Western Australia), Werribee (Victoria), and Mildura (Victoria) (see Figure 3 for locations of each growing region).

RESULTS

Global distribution of polyphagous *Liriomyza* spp. leafminers

In total, 336 unique occurrence records were compiled from published studies spanning 36 countries. The population growth model captured the known current global distribution of *L. sativae*, *L. huidobrensis and L. trifolii* (Figures 3–5), predicting strong latitudinal trends in suitability, with more tropical climates with low temperature and moisture stress generally being more favourable. Large areas currently unoccupied were predicted to support positive population growth during parts or all of the year. For example, areas of India, Europe and southern countries of the African continent were found to be suitable for all three species, suggesting these species have the potential to significantly expand their current global range (Figures 3–5). The Boyce Indices indicated a good fit to data. For all three species of leafminer we obtain indices close to one: 0.673 for *L. huidobrensis*, 0.703 for *L. sativae*, and 0.715 for *L. trifolii*. However, the models had difficulty in predicting some occurrence locations, such as the presence of *L. sativae* in Egypt and Yemen.

Australian climatic suitability for polyphagous *Liriomyza* spp. leafminers and their natural enemies

Large regions of Australia were predicted to have climates capable of supporting positive population growth for all species considered (Figure 6). The predicted distribution of *L. trifolii* is generally similar to *L. sativae* whereas the

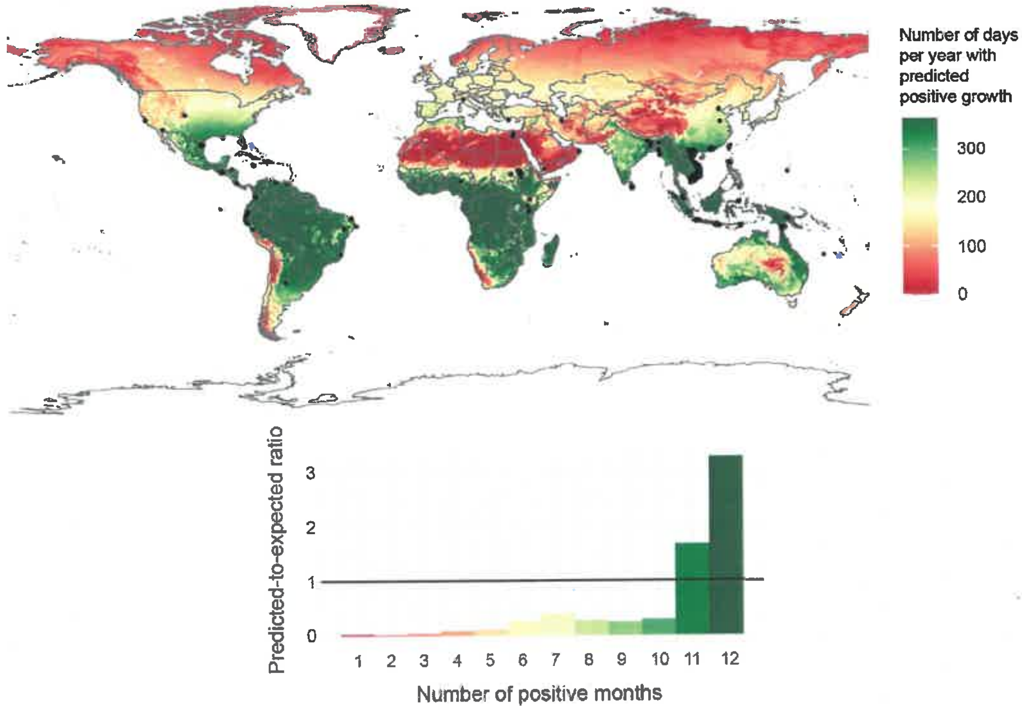

FIGURE 3 Global climatic suitability for *L. trifolii*, depicted as the number of days during which positive growth rates are predicted to be achievable using climate data from 2017. Occurrence records of *L. trifolii* (black dots) generally correspond to areas where the model predicts nearly 12 months of positive growth rate being achievable (see frequency graph inserted which shows the mean Boyce ratio by the number of months with positive growth in any given year). The Boyce Index as a measure of model goodness is 0.715, showing that the model predictions are consistent with the occurrence data.

distribution of *L. huidobrensis* is somewhat different, driven by its preference for colder temperatures and higher sensitivity to heat stress. For *L. sativae* and *L. trifolii* large areas in the north-east coastal region were predicted to support positive population growth throughout the year, whereas northern Australia possessed large regions suitable for approximately half the year. Northern regions are far less suitable for *L. huidobrensis* than for the two other leafminer species. Less suitable for *L. sativae* were the Mediterranean climatic regions of Australia (e.g., south-west) and temperate regions (e.g., south-east), which were typically predicted to be suitable between one and six months of the year. However, the suitability of these southern regions was predicted to be higher for both *L. trifolii* and *L. huidobrensis*. The arid deserts of central Australia were predicted to be unsuitable for all three species all months of the year.

Parametrising data suggested that the parasitoid, *H. varicornis* is more heat adapted than *D. isaea* (Table 1 and Figure 2). Despite these ecological differences, both parasitoids were estimated to encompass the vast majority of the predicted spatial distribution of *L. sativae*, *L. huidobrensis* and *L. trifolii* in Australia (Figure 6). *D. isaea* is predicted to have a higher persistence in southern regions of Australia, whereas *H. varicornis* is expected to persist in a wider variety of climates in northern regions. Importantly, the tip of the north-eastern Australian mainland, where *L. sativae* is presently isolated, is predicted to be highly suitable for both parasitoid species, however only *H. varicornis* has been recorded there as of yet (ALA 2022a, 2022b).

Vegetable production regions

To gain a greater appreciation of the threat *L. sativae*, *L. trifolii* and *L. huidobrensis* pose to vegetable production regions in Australia, and the potential role of parasitoids in mitigating these risks, we compared the predicted activity of each species across season and region in Australia. There was high variation in the suitability across the five vegetable production regions examined in this study (Figure 6). Analysing the impacts of seasonality in each of these regions also revealed strong monthly variation in population growth rates for *Liriomyza* spp., *D. isaea* and *H. varicornis* (Figure 7), with growth rates generally decelerating during winter or even becoming negative in more temperate regions. At Lakeland and Bundaberg, where the dominant stressor is heat, *L. sativae* and *L. trifolii* are predicted to survive year-round, although there are notable population declines predicted during the hot summer period (Figure 7). These locations are nearby vegetable and nursery production regions such as

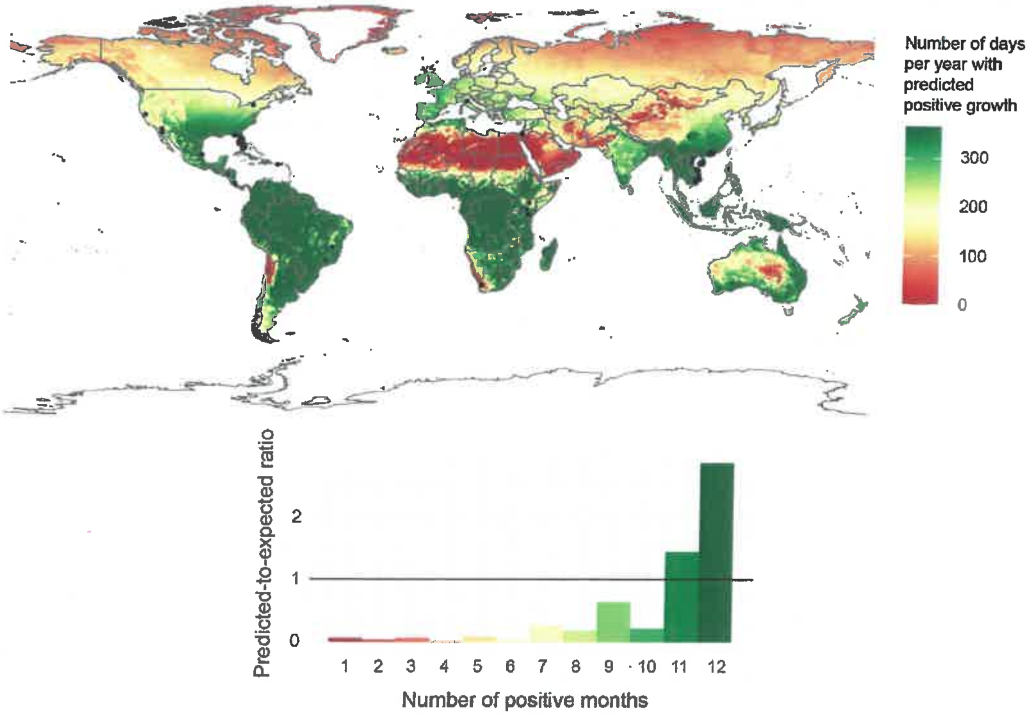

FIGURE 4 Global climatic suitability for *L. sativae*, depicted as the number of days during which positive growth rates are predicted to be achievable using climate data from 2017. Occurrence records of *L. sativae* (blue dots) generally correspond to areas where the model predicts nearly 12 months of positive growth rate being achievable (see frequency graph inserted which shows the mean Boyce ratio by the number of months with positive growth in any given year). The Boyce Index as a measure of model goodness is 0.703, showing that the model predictions are consistent with the occurrence data.

Lakeland and Bundaberg, and melon production regions such as Kununurra. *L. huidobrensis* growth is predicted to be considerably lower in these three regions, particularly in Kununurra, where survival is likely to be limited to the winter period due to summertime heat and desiccation stress. At Mildura and Werribee, population growth of *L. sativae* and *L. trifolii* is predicted to occur for approximately half of the year, with cold stress preventing survival in Mildura and Werribee in winter, and heat and desiccation stress preventing survival in Mildura in late summer. Heat and moisture stress in Mildura are also predicted to limit population growth of *L. huidobrensis* in late summer. At Werribee, *L. huidobrensis* survival is predicted survive year-round, including over the winter when population growth is expected to be very low (Figure 7). Mildura and Werribee are key vegetable production regions in Australia. High growth potentials observed across the range of vegetable production regions for *L. sativae*, *L. trifolii* and *L. huidobrensis* were generally matched or surpassed by the two parasitoids, *D. isaea* and *H. varicornis*, particularly in the three northern regions (Figure 7). *Diglyphus isaea* is predicted to survive at all locations and across all seasons, with the exception of some limits to population growth expected in Werribee in winter. *Hemiptarsenus varicornis* is expected to achieve activity year-round at Kununurra, Lakeland and Bundaberg but population growth is predicted to be prevented for considerable periods during winter at both Mildura and Werribee (Figure 7).

DISCUSSION

In this paper, we successfully modelled the potential global distributions of *L. huidobrensis*, *L. sativae* and *L. trifolii* by explicitly considering determinants of population growth from existing biological and ecological knowledge. Importantly, the models indicate there are large areas presently unoccupied by all three *Liriomyza* species with high predicted ecological suitability in many parts of the world. As of 2021, the European and Mediterranean Plant Protection Organisation (EPPO) has classified *L. huidobrensis*, *L. sativae* and *L. trifolii* as A2 pests absent from significant portions of the EPPO region whereby member countries are recommended to regulate it as a quarantine pest (EPPO 2022). Our findings confirm that *L. sativae* is at risk of establishing in Europe, and supports ongoing prioritisation of biosecurity measures, particularly during warmer months when conditions for population growth are more favourable.

In Australia, *L. sativae* (the only *Liriomyza* species currently under quarantine control), is classed under the

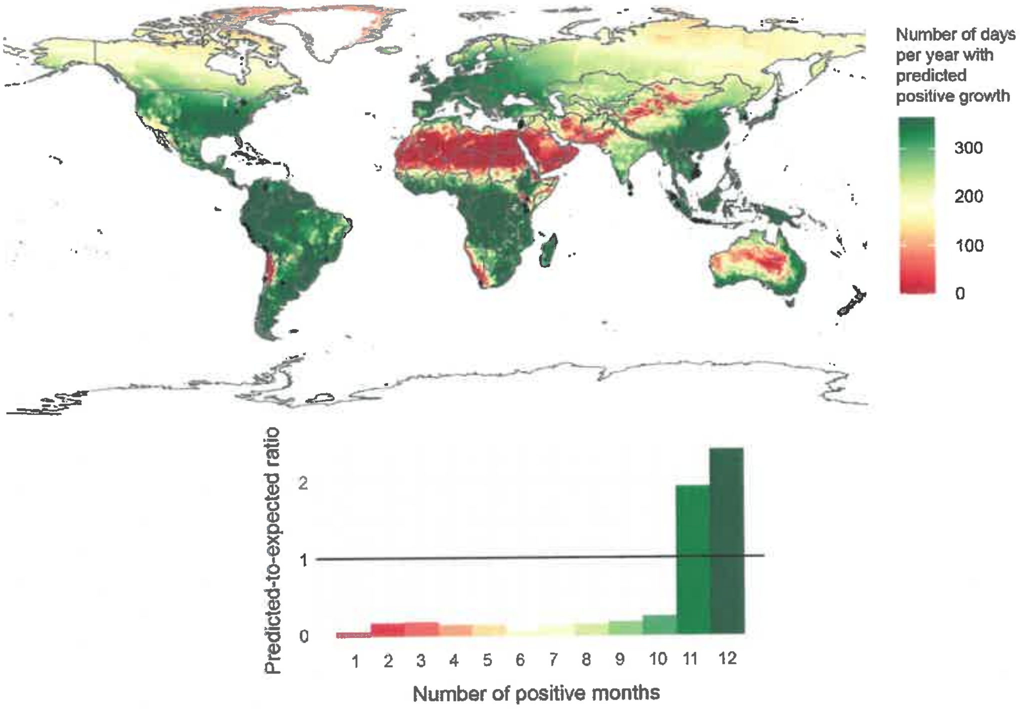

FIGURE 5 Global climatic suitability for *L. huidobrensis*, depicted as the number of days during which positive growth rates are predicted to be achievable using climate data from 2017. Occurrence records of *L. huidobrensis* (black dots) generally correspond to areas where the model predicts nearly 12 months of positive growth rate being achievable (see frequency graph inserted which shows the mean Boyce ratio by the number of months with positive growth in any given year). The Boyce Index as a measure of model goodness is 0.673, showing that the model predictions are consistent with the occurrence data.

Emergency Plant Pest Response Deed (EPPRD) as a Category 3 pest, based on the anticipated negative impact on agricultural industries and additional public costs (e.g., damage to amenities, or trade restriction) (Plant Health Australia 2022). Although *L. trifolii* and *L. huidobrensis* are now both present outside of quarantine zones, with *L. huidobrensis* already declared infeasible to eradicate, both pests are still within the early stages of their establishment within Australia (International Plant Protection Convention 2017, 2021c,b). Our modelling has highlighted many regions in Australia that have a high predicted ecological suitability but are presently unoccupied by *L. sativae*, *L. huidobrensis* and *L. trifolii*, suggesting the range of all three species is likely to expand significantly. This likely range expansion presents a serious risk to Australia's vegetable production and nursery industries. In particular, melon production regions of the Northern Territory, as well as vegetable production and nurseries across coastal regions of Queensland were identified as having high suitability for *L. sativae* and *L. trifolii*. However, there will be marked differences in the seasonal pest pressure of each *Liriomyza* species and this will affect the speed it takes these pests to realise their potential distributions within Australia. Higher annual suitability for population growth will be directly associated with higher exposure to incursion events and the successful establishment of invasive species (Maino et al., 2021). In southern Australia the predicted lower annual suitability suggests these regions will be less vulnerable to pest incursions and establishment, and are thus expected to have more time to prepare for the arrival of these pests arrive and maintain local area of freedom status. The anticipated expansion of *Liriomyza* species in regions with year-long suitability however, is expected to increase the risk of further incursions, or seasonal dispersal events, into areas with partial year suitability (Mitchell et al. 1991). Additionally, the availability of micro-climates (e.g., shaded creek beds versus open fields) will likely buffer some populations against stressful weather conditions (Kearney & Porter 2009). In many vegetable growing regions, such as Mildura, irrigation is commonplace and will likely create local areas of suitability that will provide some buffering from heat and moisture stress in late summer, as has occurred in the US states of Arizona and California (Palumbo & Kerns 1998; Smith et al. 2011; Turini et al. 2011).

The importance of micro-climates and smaller scale factors in determining the potential range of *Liriomyza* species post incursion is illustrated by the low predicted suitability of hot and arid locations like Yemen and Egypt, where *Liriomyza* spp. have been detected (Deeming 1992; Scheffer & Lewis 2005). However, a detection does not

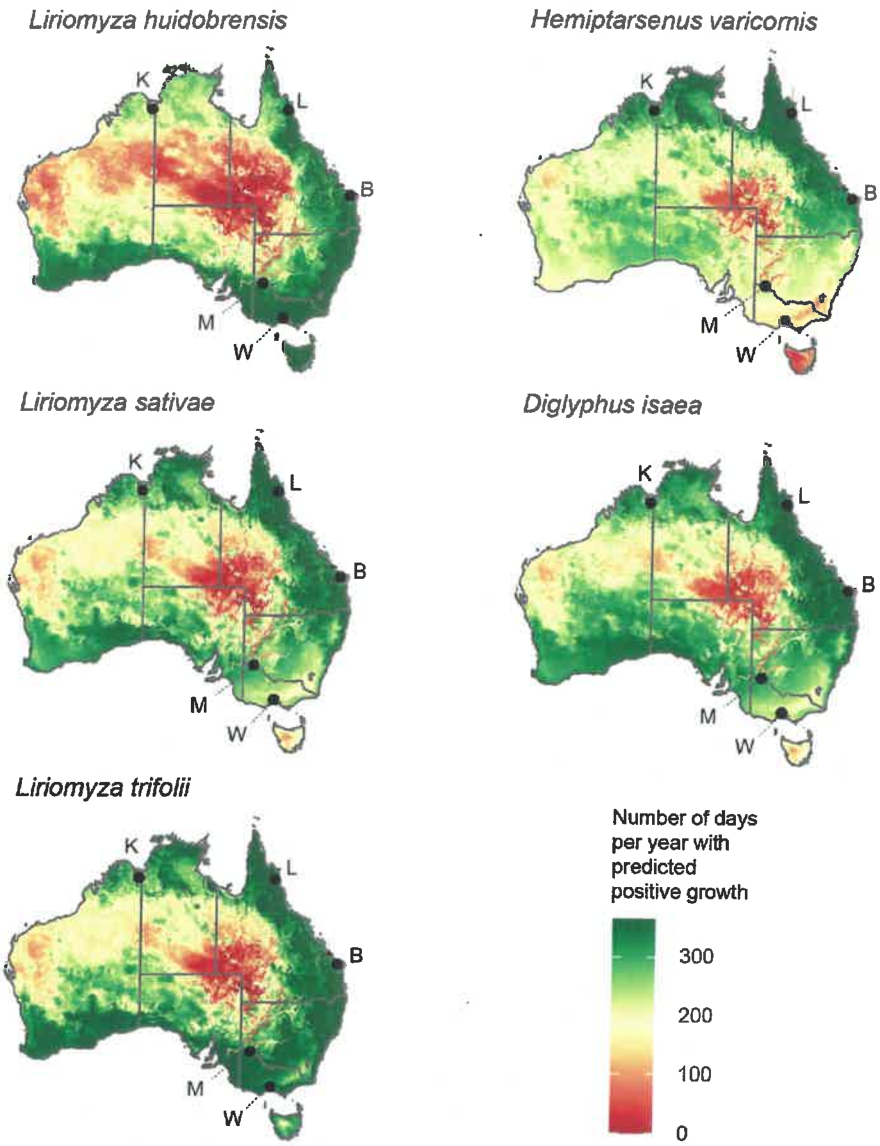

FIGURE 6 Climatic suitability for *L. sativae*, *L. huidobrensis*, *L. trifolii*, *D. isaea* and *H. varicornis* across Australia, depicted as the number of days during which positive growth rates are predicted. Letters denote the locations of key vegetable production regions in Bundaberg (B), Kunurra (K), Lakeland (L), Mildura (M) and Werribee (W).

always equate to an infestation, as is the case of Yemen, where *L. sativae* has been recorded in extremely small numbers in light traps only (Deeming 1992). Nevertheless, micro-climate factors may still allow pockets of suitability in otherwise unsuitable landscapes and thus need to considered when interpreting the model predictions developed in this study which considered macro-climatic factors. Furthermore, a successful incursion requires not only suitable climatic conditions but the availability of suitable host plants. For example, the red-banded mango caterpillar (*Deanolis sublimbalis* Snellen) has remained in the far north of Queensland for over three decades, most likely due to the lack of mango hosts in other regions (Royer 2009). For agricultural pests, a mismatch between the highest risk periods for establishment potential and the seasonality of cultivated crops could also provide a barrier to pest establishment. However, in the case of *Liriomyza* species, the most suitable climates for pest establishment often overlap with production periods for many high risk crops (HIA 2021). In addition to commercially-grown crops, the availability of wild host plants will impact a pests' ability to disperse naturally between regions and persist in production regions beyond the growing season (Karp et al. 2018). *L. huidobrensis*, *Liriomyza sativae* and *L. trifolii* attack a very wide range of non-cultivated plants (Oatman & Michelbacher 1959; Spencer 1973), including exotic weeds that are spread widely across Australia and are often associated with disturbed areas such as ports and campgrounds (Batianoff & Butler 2003). For example, during surveillance work conducted during 2018 and 2019, one of the most highly preferred weed hosts of *L. sativae*, siratro (*Macroptilium atropurpureum* [DC.] Urb.), was observed to be present growing on the fence lines of every shipping port visited, including those at Weipa, Seisia,

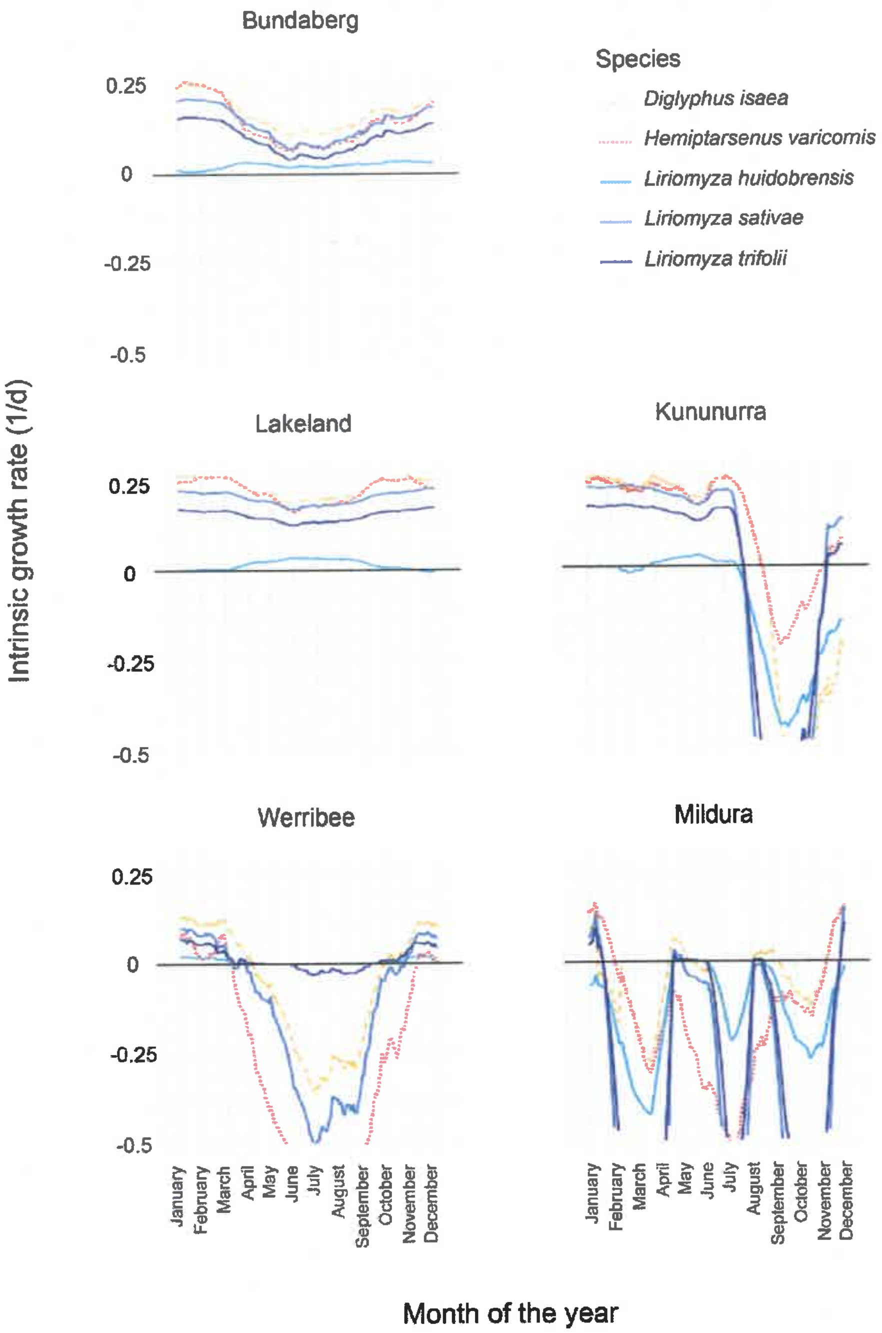

FIGURE 7 Population growth rate in five Australian location (Bundaberg (east), Kunurra (NW), Lakeland (NE), Mildura (SE) and Werribee (SE)). The curves represent a mean growth rate for each month computed on climatic data from year 2017. The growth rates result from the intrinsic rate of population growth at various temperatures using the Schoolfield equation, with parameters fitted to compiled data provided in Tables 1 and S1, and the addition of stressors: the thermal stressors (critical maxima and minima) and the moisture stress (i.e., wilting fraction) (see Figure 2).

Thursday Island, Horn Island and Cairns, and was also common within urban areas (Elia Pirtle, pers. obs.). *M. atropurpureum* is present across Australia, as is castor bean (*Ricinus communis L.*), another highly preferred weed host of *L. sativae* (Atlas of Living Australia website at http://www.ala.org.au. Accessed 5 July 2022).

The success of invading pest populations will also be influenced by existing communities of species, both through competitive interactions and biological control. For example, *L. huidobrensis*, *Liriomyza sativae* and *L. trifolii* are all recorded present in Kenya, consistent with the climatic predictions herein that all three species should be able to achieve positive growth rates for at least part of the year (Figures 3–5). However, in reality, the Kenyan population of these three *Liriomyza* species is heavily skewed towards *L. huidobrensis*, which recently was found to account for over 90% of *Liriomyza* individuals (Foba et al. 2015). *L. huidobrensis* has long tended to be the dominant of the three species at colder and higher altitudes, whereas *L. sativae* and *L. trifolii* have been expected to dominate at warmer lower altitudes (Andersen et al. 2002; Mujica & Kroschel 2011;

Rauf et al. 2000; Shepard & Braun 1998; Sivapragasam & Syed 1999; Tantowijoyo & Hoffmann 2010; Weintraub 2001). Although this previously appeared true in Kenya (Chabi-Olaye et al. 2008), *L. huidobrensis* is now displacing the other species across all temperature and altitudes in Kenya, suggesting some adaptation of *L. huidobrensis* to warmer regions (Foba et al. 2015). In Australia, it will remain unclear for some time which of these three recently established species, *L. huidobrensis*, *Liriomyza sativae* or *L. trifolii*, will dominate. Additionally, in Australia there already exists a considerable, but poorly documented community of agromyzid leafminer species, some of which show notable overlap with the preferred host plants of *L. sativae*, *L. trifolii* and *L. huidobrensis* (Spencer 1973, 1977). Moreover, these agromyzid species are known to support at least 34 genera of parasitoid wasps in Australia, many of which attack *Liriomyza* species overseas (Ridland et al. 2020). Our modelling indicates two parasitoid species expected to be important in *Liriomyza* management in Australia, *D. isaea* and *H. varicornis*, will exhibit a high degree of overlap in spatial and seasonal distribution potential with *L. sativae*, *L. trifolii* and *L. huidobrensis*. This is important given the most effective and sustainable means to control these pests has been repeatedly shown to come from parasitoid wasps (Ridland et al. 2020). Although parasitoid-prey dynamics were not considered in the models presented herein, parasitoid wasp communities represent another factor that will mitigate *Liriomyza* establishment in Australia and should ideally be incorporated into future studies (Schouten et al. 2022).

In conclusion, the models developed here have improved our understanding of how climatic conditions are likely to support and potentially restrict the distribution of *L. sativae*, *L. trifolii* and *L. huidobrensis* as they expand into novel ranges and threaten agricultural production regions. Given the early stage of the invasions and the largely unfilled climatic niche available to these three pests in Australia, our results help to highlight important vegetable and nursery production locations at greatest risk to *Liriomyza*. Biosecurity and management responses can thus be prioritised accordingly. In addition to Australia, our results highlight many regions globally where *L. sativae*, *L. trifolii* and *L. huidobrensis* have the potential to spread in the future. Within Australia, the wasps *D. isaea* and *H. varicornis* are predicted to have a large spatial and seasonal overlap with each *Liriomyza* species and thus are expected to influence the future spread of these pests. These wasp species, as well any many others not considered here (see Ridland et al. 2020), are also expected to play an important role in local pest management efforts on farm.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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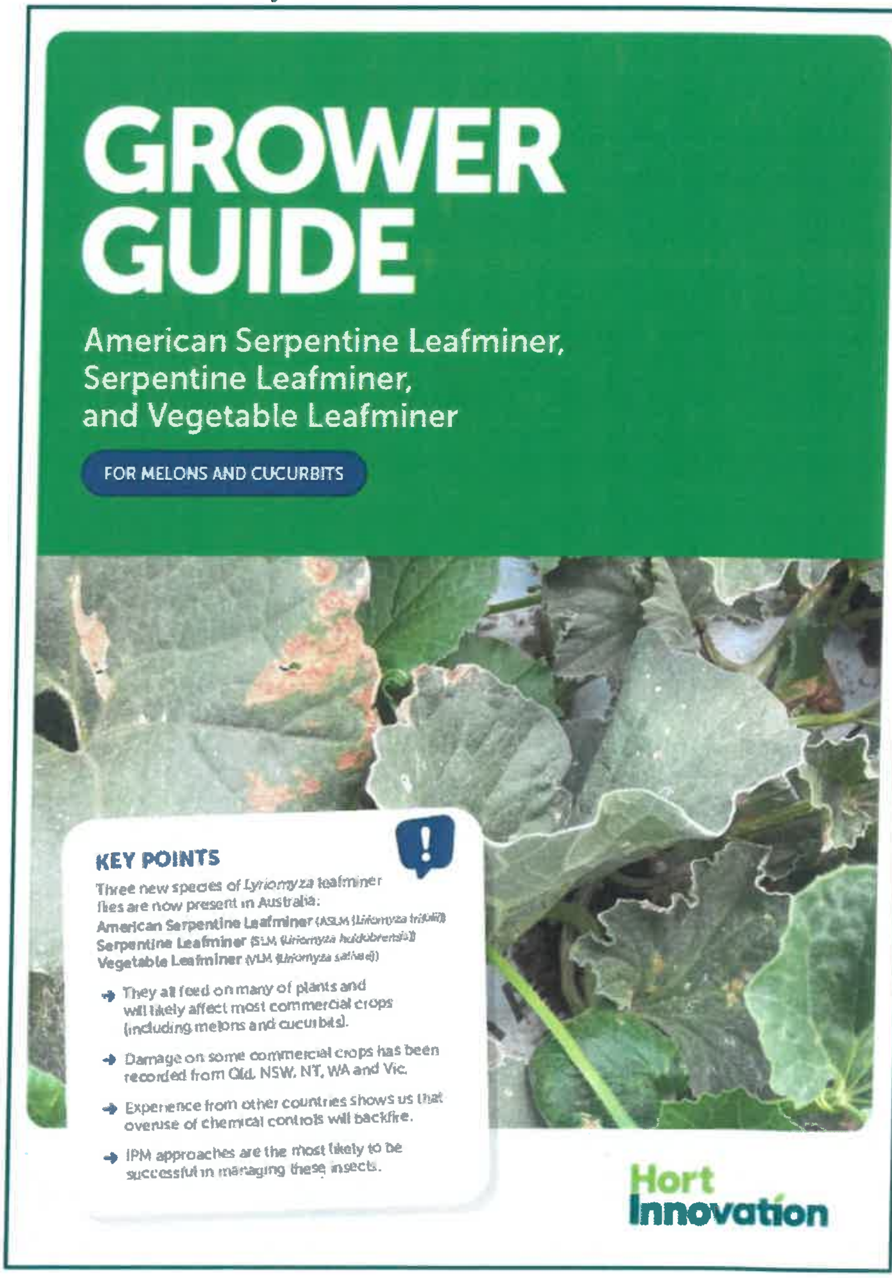
Appendix 3.1

Grower Guide for Melons and Cucurbits

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by Shakira Johnson


GROWER GUIDE

American Serpentine Leafminer,
Serpentine Leafminer,
and Vegetable Leafminer

FOR MELONS AND CUCURBITS

KEY POINTS

Three new species of *Lyriomyza* leafminer flies are now present in Australia:
American Serpentine Leafminer (ASLM (*Liriomyza trifolii*))
Serpentine Leafminer (SLM (*Liriomyza huidobrensis*))
Vegetable Leafminer (VLM (*Liriomyza sativae*))

- They all feed on many of plants and will likely affect most commercial crops (including melons and cucurbits).
- Damage on some commercial crops has been recorded from Qld, NSW, NT, WA and Vic.
- Experience from other countries shows us that overuse of chemical controls will backfire.
- IPM approaches are the most likely to be successful in managing these insects.

Hort Innovation

GROWER GUIDE

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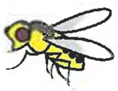
Hort Innovation

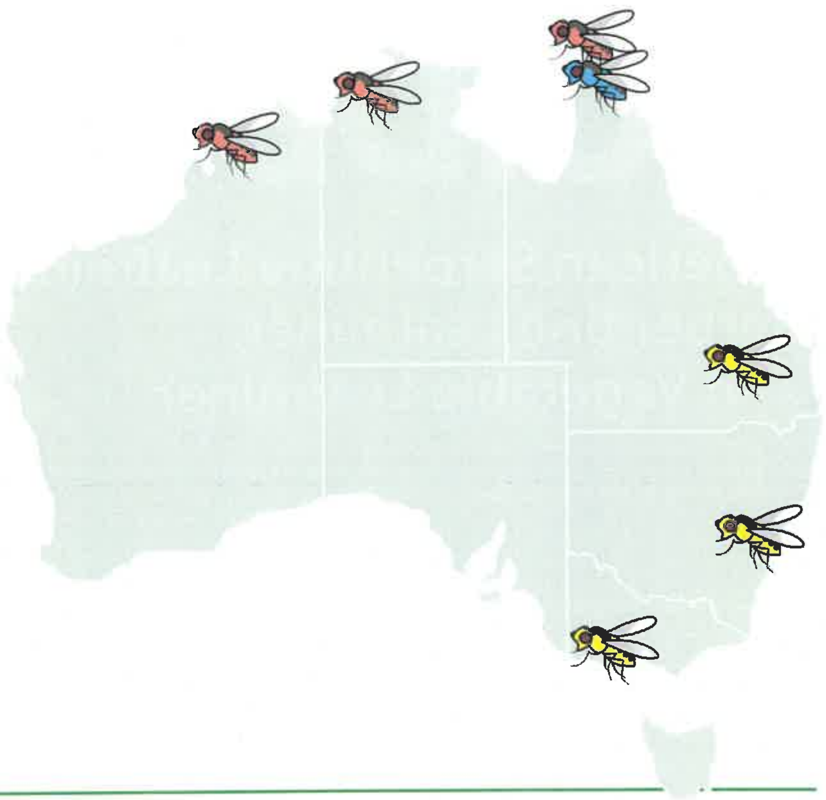
Current known distribution of the new leafminers as of 2023

Vegetable Leafminer
(first detected in Cape York, 2015)



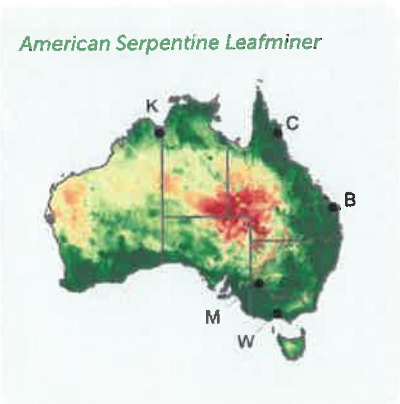
Serpentine Leafminer
(first detected in the Sydney basin, 2020)

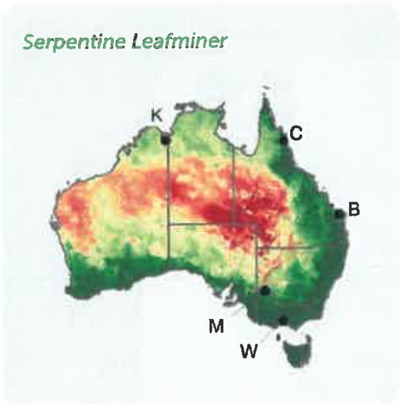
American Serpentine Leafminer
(first detected near Kununurra, 2021)

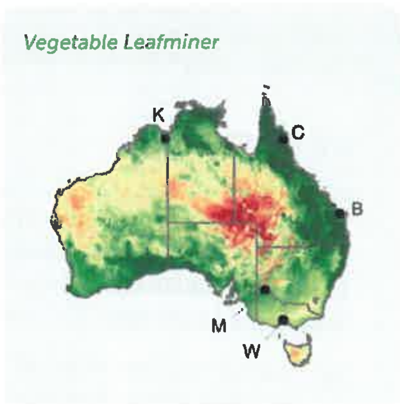


Seasonality

Each of the new leafminer species has a preferred climate suitability. Modelling has been prepared to show where and when each species is likely to be at its most active.







Number of days per year with predicted positive growth[1]

Key regions

B- Bundaberg
C- Cairns
K- Kununurra
M- Mildura
W- Werribee

1 Maino, J. et al. (2023) Austral Entomology, 62(1), 118–130.

Insect Life Cycle

Leafminers have four life cycle stages

- Typical leafminer lifecycle takes 13 to 43 days from eggs to adult emergence.
- Time taken to complete each life stage varies depending on temperature.
- Development rates become quicker as temperature increases, leading to overlapping generations.
- However, lethal temperature limits exist for each of these leafminer species:
  - ASLM 10°C and 35° C
  - SLM 5°C and 32°-35°C
  - VLM 10°C and 40°C

1 EGGS

Adult females create holes (stippling) when feeding and/or laying eggs.

2 LARVAE

These eggs hatch after 2-5 days and the larvae tunnel through the leaves creating serpentine leaf mines predominantly on the upper surface of the leaf. This is the most damaging stage for melons and other cucurbits.

3 PUPAE

The larvae then pupate, either on the leaf or in the soil.

4 ADULTS

Adult flies then emerge from the pupae, mate, and lay eggs, beginning the cycle again.

Pest & Impact


STIPPLING


LEAFMINING


PUPAE

Damage from leaf mining and feeding can cause premature leaf drop leading to sunburn of fruit, and also create points for secondary infection from fungi and bacteria.


MELON LEAVES WITH EXTENSIVE LEAFMINING AND SECONDARY INFECTION

Some naturalised and recently established leafminer flies	LOW ECONOMIC CONCERN				HIGH ECONOMIC CONCERN		
	 **Cabbage Leafminer**[1] *Liriomyza brassicae*	 **Chrysanthemum Leafminer**[2] *Phytomyza syngenesiae*	 **Beet Leafminer**[3] *Liriomyza chenopodii*	 **Bean Fly**[4] *Ophiomyia phaseoli*	 **American Serpentine Leafminer**[5] *Liriomyza sativae*	 **Serpentine Leafminer**[6] *Liriomyza huidobrensis*	 **Vegetable Leafminer**[7] *Liriomyza sativae*
MINE TYPE	Leaf	Leaf	Leaf	Leaf and Stem	Leaf	Leaf	Leaf
COMMON HOSTS	Brassicas, such as Broccoli, Chinese Cabbage, Kale and others	Sow thistle and other Asteraceae	Beets, Chickweed	Green beans and other Legumes	Chrysanthemums, Capsicum, Melons, Potatoes, and Beans	Celery, Pumpkin, Zucchini, Beans, and Potatoes	Melons, Beans, Tomatoes

1 Image credit: Dr Elia Pirtle
2 Image credit: John Duff (DAF Qld)
3
4 Image credit: Central Science Laboratory, York (GB), British Crown
5
6 Image credit: Shannon Mulholland (DPI NSW)
7 Image credit: Dr Elia Pirtle

Farm Biosecurity

How it spreads

Adult leafminers are generally considered poor flyers. The most likely cause of spread is as a hitchhiker on goods, aircrafts, vehicles, and the movement of plant material.

- Eggs and larvae may be spread via live plant material eg. cut flowers, leafy vegetables
- Pupae may be spread along with crop debris or soil or stuck on plant material at harvest

Consider which of these are relevant to your property!


Image credit: John Duff (DAF Qld)

Prevention of spread

Ensure you have a rigorous biosecurity plan in place that includes:

- Appropriate signage
- Boot sanitising stations
- Car cleaning stations
- Only purchasing farm inputs from reliable or certified sources
- Regular monitoring and surveillance of your crops
- Refusal of entry to anyone who refuses to comply with your biosecurity procedures

LEARN MORE

Information about how to maintain good on-farm biosecurity can be found online here

Download Guide

Monitoring Leafminers

- Conduct visual inspections of crops regularly, looking for stippling or leaf mining damage
- Use sticky traps to monitor for adult flies
- Visually inspect leaves to look for mines and larvae
- Inspect leaves and stems of plants for pupae that have stuck to the plant surface
- Use trays placed below crop canopies to monitor for pupae (this will only work for certain crops)

LEARN MORE

A concise guide to monitoring for leaf mining flies in Australia is available online here

Download Guide


Car Cleaning Stations


Sticky Traps


Field Surveillance

Integrated Pest Management

Foundations of an IPM approach

CULTURAL
Monitor pest and parasitoid activity to inform management decisions.

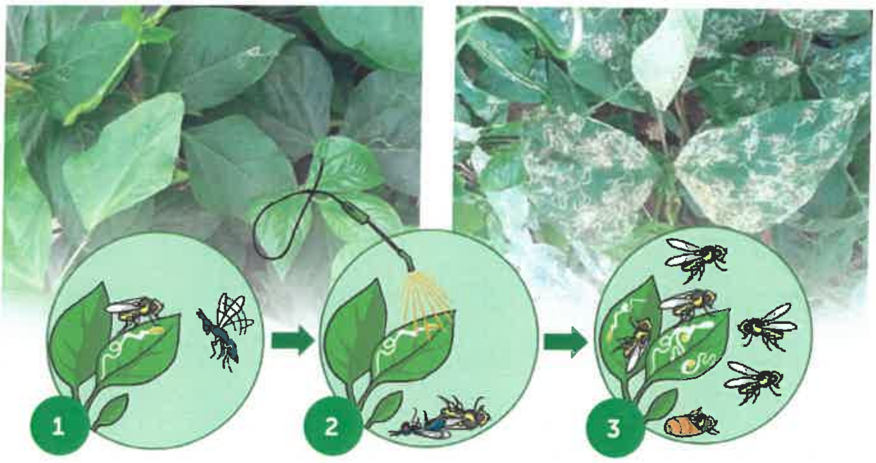
CHEMICAL
Avoid a reliance on insecticides, especially broad-spectrum products. This has lead to insecticide resistance developing and a destruction of local beneficial insect populations. Consider softer option insecticides.

BENEFICIALS
Conserve beneficial natural enemies such as parasitoids. Learn the signs of parasitism of the leafminer larvae in the leafmines to determine if visible leafmining damage is associated with active parasitoid wasps. The signs of active parasitism will indicate some control of the leafminer population by the presence of parasitoids.

Leafminer outbreaks overseas

The plant on the right was treated weekly with insecticide sprays, but only accumulated heavy damage after treatment. This is a common problem overseas, where the excessive use of non-selective and broad spectrum insecticides leads to the destruction of parasitoid wasps, which are natural enemies of leafminers. Integrated pest management programs should prioritize conservation of parasitoids and consider all chemical use in a system.

Only one of these bean plants has been treated with insecticide, but which one it is may surprise you.



1. Parasitoid wasps naturally control leafminer flies.
2. Non-selective insecticides destroy parasitoid wasps but not all leafminer flies.
3. Without parasitoids, leafminer populations can grow substantially.

Chemical management

Leafminer species have developed resistance to many insecticides.
An integrated approach is necessary to prevent further resistance.
If chemical treatments are used, rotate mode of action groups and avoid broad-spectrum pesticides. Contact, systemic, and translaminar pesticides are effective on different stages. Biological control with parasitoid wasps is more effective. Avoid harming beneficial wasp populations.

Avoid leafminer outbreaks by monitoring during high risk periods and by using softer chemicals. See table page 7.

INSECTICIDE MODES

CONTACT
Contact pesticides are effective against adults

SYSTEMIC
Systemic pesticides are effective against larvae

TRANSLAMINAR
Translaminar pesticides are effective against both adults and larvae

✕ Mortality of leafminer adult or larva

● Dispersal of chemical on/in plant tissue

Natural control by beneficials

Parasitoid wasps

Parasitoid wasps are a natural way to control leafminers. Parasitoid wasps can reach the leafminer larvae within the leaf, laying their eggs on or in the larvae. They bring about mortality through parasitism or by direct feeding on the developing leafminer larvae. Field mortality rates can reach up to 80%.

Australia has at least 50 species of these wasps that attack native and exotic pests. Four are particularly good at targeting leafminer flies:

KEY PARASITOID WASPS THAT ATTACK LEAFMINER FLIES

Opius spp.

- Larval/pupal parasitoid
- Recorded in all states
- At least three different species of the group attack native leafminers in Australia

Diglyphus isaea

- Larval parasitoid
- Present in southeastern Australia (but likely only recently established)
- Mass reared overseas for biological control

Hemiptarsenus varicornis

- Larval parasitoid
- Recorded in all states
- Important source of control overseas
- Early exploiter of new exotic leafminer

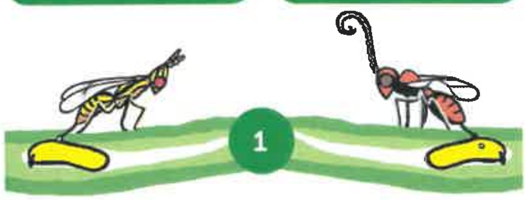
Zagrammosoma latilineatum

- Larval parasitoid
- Recorded in QLD, NSW, VIC, WA and NT
- Major source of leafminer control in Far North QLD
- Ecology and biology is poorly understood

Lifecycles of parasitoid wasps

Their lifecycles vary and can be classified as "larval" or "larval/pupal".

LARVAL	LARVAL/PUPAL



Female wasp lays egg on or in fly larva.

LARVAL	LARVAL/PUPAL
Wasp egg hatches and feeds on fly larva.	Wasp egg stays dormant until fly larva emerges and pupates.

LARVAL	LARVAL/PUPAL
After consuming the fly, the wasp pupates inside the leaf mine.	Wasp egg activates consuming pupating fly.

LARVAL	LARVAL/PUPAL
Adult wasp emerges from the leaf mine.	Wasp emerges from otherwise healthy looking fly pupa.

Look for signs of larval parasitism inside leaf mines with a hand lens (A and B). Pupae of leafminers parasitised with larval/pupal wasps will not show signs of parasitism until emergence of wasps from otherwise healthy looking leafminer pupae (C).

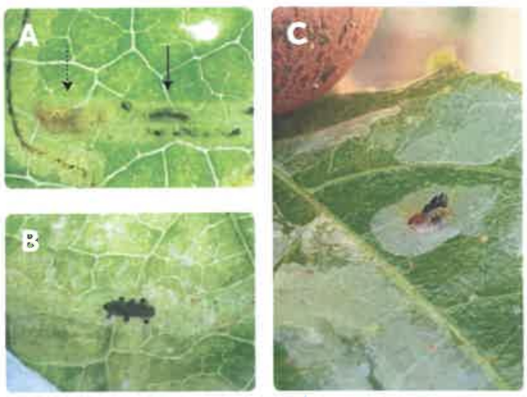


Image credit: Eddy Dunne (Total Grower Services Bundaberg)

Parasitoid wasps are much smaller than a thumb tack.

Image credit: Dr. Elia Pirtle (Cesar Australia)

Minor Use Permits Available for Leafminers† *(Liriomyza Species)*

✓ CURRENT PERMIT
O SUPPRESSION ONLY^
X CROP MUST BE DESTROYED
FC FIELD CROPPING ONLY

Active Ingredient	Cyromazine	Chlorantraniliprole	Cyantraniliprole		Spirotetramat	Spinosad	Spinetoram		Abamectin	Emamectin Benzoate	Dimethoate	Thiamethoxam & Chlorantraniliprole
Mode of Action	17	28	28	28	23	5	5	5	6	6	1B	4B & 28
Activity	Translaminar	Systemic	Systemic	Systemic	Systemic & Translaminar	Contact & Systemic	Contact & Translaminar	Contact & Translaminar	Contact & Translaminar	Translaminar	Contact & Systemic	Systemic
Example Product	Diptex 150WP	Coragen	Benevia	Benevia	Movento 240 SC	Entrust	Success Neo	Success Neo	Vertimec	Warlock	Dimethoate 400	Durivo
Permit Number	PER81867	PER87631	PER90387	PER90927	PER88640	PER90928	PER87878	PER91155	PER81876	PER87563	PER89184	PER91161
Expiry	30/09/2024	30/06/2024	31/12/2023	31/12/2023	29/02/2026	30/04/2024	31/12/2027	30/06/2024	30/04/2024	30/06/2024	31/03/2025	30/06/2024
Impact on Beneficials including parasitoids	LOW	LOW	LOW	LOW	LOW TO MOD	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	HIGH	HIGH
Brassica Veg*	X							✓		O		
Broccoli	✓							✓		O		
Bulb Onions									O			
Bulb Vegetables			✓						O			
Cabbage (Head)	X							✓	O	O		
Capsicums & Chillies			✓		O	✓		✓	O			
Celery				✓	O FC	✓		✓	O			
Corn			✓					✓				
Culinary Herbs						✓		✓				
Cucurbits	✓		✓			✓		✓	O			
Eggplant			✓		O	✓		✓	O			
Fruiting Veg#	✓		✓			✓		✓	O			
Snow & Sugar Snap Peas	✓				O	✓	✓		O			
Green Beans	✓				O FC	✓	✓		O			
Green Peas	✓					✓			O			
Leafy Brassicas	X					✓		✓				
Leafy Vegetables+	X					✓		✓	O			✓
Legume Vegetables	✓					✓			O			✓
Lettuce (Head)	✓				O Inc Leafy	✓		✓				
Parsley					O	✓		✓				✓
Potatoes			✓			✓		✓				
Pulses	✓					✓			O		✓	
Rhurbard					O FC	✓		✓	O			
Root & Tuber Veg	✓					✓		✓	O			
Silverbeet & Spinach	X	O				✓		✓	O			✓
Stalk & Stem Veg	✓					✓		✓				
Tomatoes			✓		O	✓		✓	O			

Disclaimer: This is a quick reference guide and omits certain elements included in minor use permits, such as jurisdictions and restraints. Every effort has been made to provide the most complete and up-to-date information as of publication date, however, we recommend you check the specific details on the APVMA website in the hyperlinks provided.

† Current as of publication date.
* Excluding Broccoli
\# Excluding Cucurbits, Corn or Mushrooms
\+ Excluding Lettuce

^ Suppression denotes a level of effectiveness less than total control but still of economic benefit.

Trade & Movement Restrictions

There are currently movement restrictions in place to limit the spread of leafminers in Australia. Interstate trade regulations are updated regularly. Always check for the most current information with your relevant state government department.

FAR NORTHERN QUEENSLAND

Vegetable leafminer is a declared far northern QLD pest and is limited by the movement restrictions of the far northern biosecurity zones.

Learn More

WESTERN AUSTRALIA

In Western Australia movement of material that could potentially carry American Serpentine leafminer is restricted from the Shires of Broome, Derby West Kimberley, and Wyndham-East Kimberley into the rest of the state.

Learn More

Reporting Requirements

Some jurisdictions have legal requirements to report the detection of leafminers. You can report pests by calling the Exotic Plant Pest Hotline on 1800 084 881

STATE	VLM	SLM	ASLM
NSW	Reportable	Not Reportable	Reportable
NT	Reportable	Reportable	Not Reportable
SA	Reportable	Reportable	Reportable
QLD	Reportable	Not Reportable	Not Reportable
TAS	Not Reportable	Reportable	Not Reportable
VIC	Reportable	Not reportable	Reportable
WA	Reportable	Reportable	Reportable

Regardless of the legal requirements in your region, if you suspect a pest not currently known to be in your area, please take photos of the pest and call the Exotic Plant Pest Hotline on 1800 084 881

Acknowledgements

Management strategy for serpentine leafminer, *Liriomyza huidobrensis* (MT20005) is a strategic levy investment under the Hort Innovation Vegetable, Potato – Fresh and Potato – Processing, Onion and Melons Funds.

This project has been funded by Hort Innovation using the vegetable, potato, onion and melon research and development levies and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Other Resources

MANAGEMENT OF LEAFMINING FLIES

A more in-depth guide to the management of leafmining flies is available here.

Download Guide

Hort Innovation MELON FUND

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Hort Innovation VEGETABLE FUND

Appendix 4.1

Northern Australia Extension Activities Report

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

By Greg Owens

MT20005 Northern Australia Extension Activities Report

Introduction

In May 2023 an independent horticulture extension consultant was engaged by AUSVEG to complete their extension and monitoring & evaluation component of the MT20005 Leaf Miner project. The original project was scheduled to be completed by March 2023, but an extension was gained until November 2023 to complete outstanding components of the project, including up to 12 workshops across the vegetable growing areas of Northern Australia and to complete updated extension materials.

This project, MT200005, was a biosecurity exotic leaf miner incursion response project and aligned closely with the AUSVEG biosecurity program across all states and vegetable commodities. It followed the biosecurity exotic incursion leaf miner preparedness project MT16004 that had produced a wide range of documents to inform growers of the dangers of the exotic leaf miner flies and the most successful approaches from overseas experience to managing them in a commercial situation.

The workplan for this part of the project called for workshops, or appropriate extension activities such as farm visits, in NT, Nth Qld and Nth WA. The team leader from Qld DAF, with AUSVEG, had conducted a workshop for agronomists in the Granite Belt in December of 2022 and this became a blueprint of the workshops to be rolled out across the North. A typical running sheet for the workshops is attached at the end of this extension report. Advice and engagement were sought from members of the project team across Australia, local farming Associations, local VegNET officers, relevant State, Territory and Federal government agencies and key local growers.

The workshops also incorporated a Monitoring and Evaluation (M&E) Survey for growers and a separate but closely aligned survey for industry participants to gauge the awareness and use of current resources and a set of discussion questions to investigate attitudes and intentions for practice change. Copies of these instruments can be found in the M&E report.

Northern Australia MT20005 Extension Activities

Attendance at extension activities

Location	State	Date	Event	Attendance/ participation		Comment
				Growers	Industry	
Granite Belt	Qld		Workshop	Unknown	Unknown	Agronomists Q DAF team
Darwin Rural	NT	4/4/2023	Workshop	8	4	Combined Veg growers WS
Hort Connections	SA	6&7/6 2023	Conference	N/A	N/A	AUSVEG stand
Darwin	NT	6/7/2023	Workshop	3	10	Growers and Agronomists
Cairns	Qld	14/7/2023	Stallholders	5 stalls		Rusty's Markets Hmong growers
Innisfail	Qld	15/7/2023	Workshop	10	1	Growers shed Hmong
Ayr	Qld	18/7/2023	Farm Visits	2		Large Veg and fruit growers
Ayr	Qld	18/7/2023	Workshop	1	7	Grower and Agronomists
Bowen	Qld	19/7/2023	Workshop	0	14	Agronomists
Geraldton	WA	8/8/2023	Farm Visits	4		Cucumber protected cropping sites
Carnarvon	WA	10/8/2023	Farm Visit	1		Protected cropping capsicums
Carnarvon	WA	10/8/2023	Workshop	40 farms	12	Viet Growers and Industry
Broome	WA	16&17/10/2023	Farm Visits	5	4	Small mixed farms and TAFE
Kununurra	WA	19/10/2023	Workshop	5	8	Major growers and Ord Co
Gatton	Qld	1/11/2023	Field Day			600+ attendees
Bundaberg	Qld	7/11/2023	Farm Visits	3		Large Chilli growers
Bundaberg	Qld	8/11/2023	Workshop	0	19	Agronomists and Industry only
Total				84	60	144 attendees

MT20005 Extension Activities Report

Darwin Rural Workshop

The Darwin Rural workshop was held in a fruit and vegetable freight consolidator's shed in the Darwin Rural vegetable growing area 45km south-east of Darwin. The event was coordinated by the NT VegNET officer and was attended by vegetable growers and industry support staff. 15 growers and industry support staff attended the workshop. American Serpentine Leaf Miner (ASLM; *Liriomyza trifolii*) was one of the topics and was presented by the yet to be engaged consultant. ASLM was detected in the NT in 2021 and had caused some damage on early season long and snake bean crops and was found in a range of crops and weeds across the whole Darwin rural area.

The consultant used the existing extension material from MT16004 and with the NT DITT support material highlighted the importance of IPM programs in managing ALSM. This approach was well accepted as the region was embracing an Integrated Pest Management (IPM) approach to cluster caterpillar (*Sodoptera litura*) that was causing almost complete crop failure of the okra and snake bean crops and was highly resistant to registered chemicals. This approach was consistent with the IPM response to the Fall armyworm (FAW; *Spodoptera frugiperda*) incursion that was found in the NT in 2020.

Parasitoids had been found on ASLM in the NT by the MT20005 project team at NT DITT which explained the minimal damage to crops across the vegetable and melon growing regions. This fits well with the developing farm pest management system where the beneficial insects that control the Spodoptera species were found to be effective when managed with an IPM approach. The key focus of the presentation was to reinforce the messages from the **Management of leaf mining flies in vegetable and nursery crops in Australia** guide published in the preceding project MT16004 that ASLM would only be a problem through over spraying and use of broad-spectrum insecticides. No formal evaluation was conducted as the consultant was drafting appropriate surveys and discussion questions but the clear response from the group was that this approach would fit with their current practices. This became the mantra for all further engagements.

Key Learnings: Growers in the Darwin Rural region know what to monitor for and that their IPM program will manage exotic leaf miners in their crops. This supported the current industry practice of changing momentum towards IPM as a way of building resilience to insect pests on their farms.

1 Darwin Rural Workshop

2 Darwin Rural Workshop

Hort Connections

The consultant attended Hort Connections on 6 and 7 June 2023 and was on the AUSVEG stand for several sessions and engaged with growers and industry from across Australia. The extension material from MT16004 was available and used as the focus for discussion on the exotic leaf miner's management.

This proved beneficial as the consultant was able to correct misunderstandings and improve awareness of the exotic leaf miners that were now in Australia in several vegetable and melon growing areas. There was a view from WA that potatoes were not badly affected which was contrary to the experience of potato growers in the Lockyer in Qld. The consultant and the AUSVEG biosecurity staff used this opportunity to engage with the VegNET officers to inform them of the workshops in some of their areas and to ensure local participation in the vegetable and melon growing regions.

Key Learnings: Forward planning for the workshop and farm visits across northern Australia and increased awareness across jurisdictions of the impact of the exotic leaf miners in affected communities.

Darwin Workshop

The Darwin workshop was conducted on 6/7/ 2023 at the NT DITT conference rooms and was modelled on the granite belt workshop conducted by the project leader from Qld DAF in December 2022. The facility enabled the setup of a microscope with video camera and the individual microscopes would be available for attendees to use looking at the leaf miner pests and parasitoids. Biosecurity was the underlying theme and NT growers who have been through a series of recent pest and disease incursions were aware of the likely incursion pathways.

The presentations supplied by the members of the project team from Qld DAF, NT DITT, University of Melbourne, NSW DPI and AUSVEG contained very detailed scientific information produced by the project along with short videos that gave great insight into the pest and its management. The IPM theme was very strong and the update on FAW reduced impact, pest management, parasitoids and chemical resistance complemented the key message of increased use of IPM to manage these exotic pests. The APVMA approved permits table for the exotic leaf miners was provided to give some guidance on which chemicals still had some effectiveness and the likely impact on beneficials within their growing systems.

A formal Monitoring and Evaluation (M&E) process was conducted during this workshop with participants answering a survey during the practical sessions on current awareness and use of extension resources and then participating in a table discussion process and supplying written answers to a set of discussion questions used to look at future intentions. This M&E was completed at all activities conducted after this workshop, adapted to suit individual farm visits, large groups, and non-English speaking background (NESB) growers as rich interviews or large group responses.

Key Learnings: Participants could see the pests and parasitoids and understand the size of the species involved. The permits table provided guidance on using chemicals within an IPM program. Clear resistance data showed the range of chemicals likely to be counterproductive. Practice change towards IPM was clearly evidenced in the discussions as part of a whole of farm pest management and building resilience approach. This workshop reinforced that the two recent incursions of ASLM and FAW were intrinsically linked to the growers understanding of how they could respond and manage their farms.

3 Darwin Workshop practical session

4 Darwin Workshop Local growers in practical session

Far North Queensland (FNQ)

Rusty's Markets

Rusty's markets is a fresh fruit and vegetable market located in the CBD in Cairns and the stall holders are predominately from a non-English speaking background and access a significant amount of their produce from small market gardens in the Cairns, Yarrabah, Atherton Tablelands, and Innisfail regions of FNQ. The stalls are a mixture of local producers selling directly to the consumers and local small merchants who buy and sell local and southern fruit and vegetables. This market is very significant for biosecurity as the links to the quarantine areas of Cape York are through Cairns Airport and the tourist and community traffic from the Cape. The range of Asian leafy vegetables, fruiting vegetables, herbs and spices, root vegetables and weeds make these farms ideal sites for surveillance.

NT Farmers has projects with the North Australian Quarantine Strategy (NAQS) team in Cairns and Darwin to engage with NESB communities in the far North such as the Vietnamese and Cambodian background growers in the NT, to enhance their surveillance coverage and to build awareness within these communities to biosecurity practices and threats. The key community in Rusty's markets are the Hmong people from northern Laos and bordering countries and don't have a specific written language and were completely off the radar to both NAQS and the local FNQ DAF horticulture team. The project team visited Rusty's Markets in Cairns on 14/7/2023 to reengage with these families and participated in a sepak takraw sport community event to build stronger relationships with the younger Australianised Hmong generation.

Key Learnings: Identified additional commercial Hmong vegetable growers in the group outside Innisfail near South Johnstone Research Station. Invited these additional grower families to participate in the workshop that followed in the growers shed in South Johnstone. Updated NAQS on the current strategies for managing exotic leaf miners once they have got past their border protection programs, including the importance of beneficial insects such as the parasitoids. Improved NAQS engagement with local market garden growers.

5 Stall holders and NT Farmers discussing Innisfail Workshop at Rusty's Markets

6 Some of the team at Rusty's Markest

Innisfail Workshop

The 15/7/2023 workshop with the Hmong growers was a more low tech event with some presentations in the growers' shed, a short practical on soil health and building organic matter in the alluvial and deep red soils. Discussions about the resources available for exotic leaf miner when they arrive and managing FAW along with the resident cluster caterpillar were included. Growers attended the workshop and one long term local tropical horticulture officer from Qld DAF from the South Johnstone Research Station.

The growers were extremely information hungry and quoted their only source of information as the local resellers and they were often supplied with the cheapest broad-spectrum chemicals to "kill everything" on their crops. This resulted in a constant spray regime with chemicals that were devastating on any beneficials. Their farming system had no resilience, and while surrounded with the best biodiversity and extensive bio-refuges along the South Johnstone River in Australia, were not aware of any beneficial insects and what to look for. This was evident in the impact of Cluster caterpillar on their taro. Some more progressive farmers were trying to search the internet but found very little relevant to tropical vegetable production and struggled to implement better practices. They now have the links to the leaf miner and other information on the AUSVEG website and other relevant sites, as well as the contact details for the local QLD DAF horticulture team at the research station.

The leaf miner extension materials were gladly accepted, and we discussed them at length. The permits table did not just give them options for when the pest arrived but opened their eyes to all the possibilities of using softer more targeted chemicals to control their pests and to build their populations of beneficials within their farms. The discussion around resistance also enabled the growers to understand the amount of spray failures and the constant need to increase chemical rates.

The growers were experimenting with pea straw mulch but had applied it to their rich red soils near the shed, whereas it was more needed on the heavier clay soils on the river flats that were compacted and overworked. The mulch was very damp, and it was possible to squeeze humic acids out of the mulch by hand to show the source colour of organic carbon compounds that would improve their soils. Adding some of these compounds to the clay soil sample increased its friability almost instantly.

Key Learnings: The growers are now connected to relevant information sources and the local department. They have been exposed to the basics of IPM to manage their current pests and FAW and to build resilience to the possible leaf miner incursion. The growers were given information on the soil health resources and are moving towards better soil management. The growers are now willing participants in future surveillance activities by both NAQS and QLD DAF.

7 Innisfail Workshop

8 Discussing Soil health at Innisfail Workshop

Burdekin

Ayr Farm Visits

The team visited two major vegetable and melon farms in the Ayr district on the morning of the 18/7/2023. Both growers were aware of the exotic leaf miner fly incursions in the Cape, but both said they would refer to their agronomists for advice if, and when, it became a problem in their area. The eggplant and tomato grower did note that there was a leaf miner in his eggplants but on inspection it was a much larger caterpillar, and the sample was identified as a common local pest. Potato leaf miner.

The growers were not going to attend the workshop as it was peak season with harvest in full flow. They did appreciate the visit and spent time talking through the pest management approaches and their biosecurity issues. One farmer is on a main arterial road and related how passing traffic especially tourists would often stop and walk into his paddocks and help themselves to vegetables. Despite the theft and the associated biosecurity risks from vehicles that may be returning from Cape York he was reluctant to fence the blocks quoting cost and convenience for machinery.

Key Learnings: The farm visits allowed the team to scout for suspicious leaf mines in an unaffected area and remind growers that weeds are a common host. The visits reinforced a focus on farm biosecurity with the growers and increased the understanding of the team on how whole of farm management often involves compromise but can change as different pressures impact on production.

9 Ayr Farm Visits

10 Ayr Farm Visits collecting samples for the Workshop

Ayr Workshop

The Ayr workshop was held at the Ayr research station on the afternoon of 18/7/2023 and involved the largest team to deliver the workshops up to this date, with the Team leader from QLD DAF and the local DAF entomologist from Bowen, AUSVEG, NT Farmers and University of Melbourne as well as the consultant all presenting. This workshop followed previous leaf miner workshops that were delivered as part of MT16004 but with different participants. The consultant had attended the previous workshop and supported Vietnamese growers from the upper Haughton region to attend, however harvest pressure impacted on their ability to attend this workshop. There was very little contribution from the local VegNET officer who did not attend the workshop.

The research station was set up with microscopes from Bowen and the video camera microscope that was well received by the participants. This was used to look at other small pests found on leaves in the local area including a leaf mining beetle larvae that left very similar tracks to the exotic leaf miner flies.

The reseller agronomists who attended from one company were very new to the job. The Team leader had only two years' experience and was unreceptive to the IPM message, with the view that they had to spray all crops for a range of pests, and any restraint on that would impact on their crop protection programs with their large company clients. Another company agronomist who works with the Vietnamese growers was much more receptive and continues to assist growers in a more sustainable approach.

The update on chemical resistance of the exotic leaf miner and FAW was discussed, and the permits table was well received.

Key Learnings: This workshop left the team with mixed feelings and a concern that young agronomists within the reseller commercial framework were unreceptive to encouraging beneficial insects in an IPM approach and would continue with their current practices even when the exotic leaf miner make their way to the upper Burdekin.

11 Ayr Workshop practical session

12 Ayr Workshop showing video microscope

Bowen Workshop

The Bowen workshop was conducted at the Bowen Research Facility on 19/7/2023. It was the best attended workshop so far, with agronomists and industry participants representing major vegetable and melon companies, private agronomists and suppliers, departmental officers, and resellers. There was a high level of understanding of the exotic leaf miner information and FAW that was a legacy of previous workshops from MT16004 and farm visits. There was minimal involvement by the local VegNET officer with only a brief appearance prior to the workshop starting.

Participation in the presentations was active, with insightful questions to presenters on the new developments in diagnostics, local parasitoids detection and management, along with chemical resistance work in both exotic pests. Their teams have been actively managing FAW for several seasons and were confident they could identify the pest from cluster caterpillar at the first instar from its behaviour. They questioned the use of the LAMP in the field for FAW but were interested in the eDNA diagnosis of old leaf mines for the exotic leaf miner pests.

There was strong support for IPM approaches to managing the exotic pests which would integrate with their current pest management practices. The level of experience in the room was much higher than in Ayr, and the capacity to discuss and consider different concepts on pest management was amazing. The level of discussion of the permits table showed that most participants had experience with these and other chemicals and how they fitted within their IPM systems and the likely collateral damage on the local beneficials.

There was also an atmosphere of shared respect in the room from the participants, both researchers and industry members due to the years of experience in vegetable and fruit production present.

Key Learnings: Bowen demonstrated a very high level of understanding of the issues around any incursion of exotic leaf miners into their region, and a clear concept on how it fitted with their current pest management practices. This suggested that drastic practice changes would not be required, and any incursion could be managed with fine tuning of existing practices.

The workshop clearly demonstrated the value of ongoing engagement with industry representatives in growing regions so that the following extension activities build on the existing expertise and knowledge not starting from scratch as we seemed to do in Bowen. The difference between Ayr and Bowen was stark even though they are in the same growing region.

13 Bowen Workshop presentation by team leader

14 Discussing parasitoids at the Bowen Workshop

Western Australia

The program for WA comprised of two tours with the first going to Geraldton and Carnarvon via Perth and a later trip to Broome and Kununurra from Darwin. The Geraldton and Carnarvon activities were highly focussed on the Vietnamese growers in both areas and included the key biosecurity messages from AUSVEG, Vegetables WA and the NT Farmers NESB engagement project with NAQS. The Geraldton and Carnarvon activities received great support from Vegetables WA with the WA VegNET officer and the Vietnamese engagement officer travelling with the team and participating in all these activities.

The Broome and Kununurra activities also had a biosecurity theme but a much larger focus on IPM as both centres have ASLM and FAW incursions. The farm visits in Broome were coordinated by the local DPIRD horticulture group and had less of the team presenting due to costs and injury to team DPIRD staff members.

Geraldton Farm Visits

The team visited four farms in the Geraldton area rather than run a formal workshop, on the advice of the local DPIRD officers and following discussions with a local grower as the dozen Geraldton cucumber growers were in peak production period. Cucumbers in protected cropping is the only large-scale vegetable production in the area with Geraldton being a major field crop and livestock centre.

The local DPIRD biosecurity officer was also keen to discuss the monitoring for exotic leaf miner species and for the project team leader to check positioning of the traps. Cabbage leaf miner (*Liriomyza brassicae)* is established in the area and only impacts on brassica species. One specimen was found in the wild radish weeds in the garden of the motel. Monitoring for other Liriomyza species is a key activity as ASLM is present in the North and movement restrictions apply to plant material from the far north of WA as well as for other infected states. FAW was not an issue for the cucumber growers but would presumably impact on the field crops in the area.

The cucumber growers visited were aware of leaf miners as a problem but had seen no evidence of mines on their cucumber leaves, so it did not rate highly on their radar. Their pest management systems were geared to protected cropping and concepts such as release of parasitoids and other beneficials was well understood. Where the cucumbers were grown in ground there were a significant number of weed hosts for the Cabbage and exotic leaf miner flies.

One grower was using hydroponics as a more sustainable option as soil health deteriorates over time in a fixed protected cropping system. His system was more complex, and he had advice from specialist agronomists from southern WA. Yellow sticky trap tape was used both as a monitoring and a pest management tool in one of the farms visited. This was accompanied by the planting of beneficial host plants, such as flowering basil, to maintain beneficial populations.

Key Learnings: The team leader could provide advice to fine tune the placement of traps for monitoring the exotic and established leaf miner species. Growers were made aware of the documents available to help manage exotic leaf miner incursions from the AUSVEG website and support from Vegetables WA VegNET and engagement officers. The key message was, if suspicious mines were found on the cucumber leaves, they need to be reported to the local DPIRD officers and tested.

15 Protected Cropping cucumbers in ground

16 Protected cropping hydroponic cucumbers with yellow sticky tape and flowering basil

Carnarvon Farm Visit

The team had one formal farm visit during the trip to Carnarvon to a protected cropping chilli and capsicum grower. The grower and his wife were of Vietnamese background and had excellent English. They were part of the next generation of Vietnamese growers in the area and had a reputation for excellent quality capsicums and chilli and sold to the Perth market. They also had regular contact with Vietnamese growers in the Darwin area and prior this assisted with planning the farm visit.

Their knowledge of the exotic leaf miners was very rudimentary with some knowledge that it was "up North". Their pest management was very effective but highly chemically based. It was surprising to find an active Spined predatory shield bug in the small remaining uncovered planting of chilli. This beneficial is critical in the control of cluster caterpillar and will most likely also be effective on FAW. This bug was collected and paraded on the big screen at the workshop that evening in Carnarvon.

They examined the guides from MT16004 and were given links to the AUSVEG leaf miner page. Like most Vietnamese growers they are very tech savvy on their phones with most of their commercial dealings done through their smart phones and were comfortable accessing information that way. The permits table was accepted and some chemicals on the list were in their spray program. There was no desire to change current practices that were producing excellent yield and quality.

Key Learnings: This grower has a system that is working very well currently and has no need to change practices. They are more aware of the possible impact of exotic leaf miner flies but would deal with that when it arrives.

17 Outside chilli planting where Spined predatory shield bug was found

18 Excellent capsicums from in ground protected cropping

Carnarvon Workshop

The Carnarvon Workshop was held in a growers shed on the evening of 10/8/2023. It was a strong collaborative effort between DPIRD, Vegetables WA, AUSVEG and NT Farmers and hosted by the

Carnarvon Vietnamese Community Association. The event was held later in the day so that the maximum number of growers and support personnel could attend. The event had a community focus and was supported by locals cooking a BBQ during the workshop and regular traffic to and from the drinks fridge. For those that are familiar with large Vietnamese gatherings for any reason, this is an essential part of interacting with their community. Translations into Vietnamese were provided by the Vegetables WA engagement officer who also presented on MRL's, which gave a good linkage into the permits table, and by the local DPIRD research officer also originally from Vietnam.

A total of 95 participants signed the attendance sheet which we believe represented about 40 farms and 12 industry people, with wives and children a key part of the night. Growers were especially interested in viewing the samples of the leaf miners and parasitoids through the microscope and camera, with the wives being the most active in participating in the practical sessions. Samples of local pests and the one predatory bug found also received close attention under the microscope and on the big screen.

The growers have very intense pest management programs, which are supported by the local Carnarvon Growers Association Co-op (CGA). CGA provides advice and products. They have had a resident agronomist in the past but are struggling to fill this gap. This leaves the growers very information hungry and looking for regular input. Vegetable WA has also traditionally provided advice and support to these growers through their previous VegNET officer and have now employed their Vietnamese Engagement officer to continue this work.

The exotic leaf miners have not yet been detected in Carnarvon, so the discussions were "what if" and "when" with a lot of interest in the permits table and the management guides. There was a very clear message that these need to be provided in Vietnamese language. The discussions around how their management may need to change was robust with a clear preference to maintain a chemical-based system within the protected cropping systems. The amount of open field vegetable crops is reducing but still has significant areas of eggplant, tomato, pumpkin, and melons. The Gascoyne River provides a bio-refuge for beneficial insects and runs the full length, down the middle of the farming area. Previous studies have looked at the opportunities for IPM systems for vegetable growers in the Carnarvon district but have had no widespread uptake.

Key Learnings: This community welcomes information from projects and the growers seek to understand how to produce good quality produce. They feel the systems they have are working well and are comfortable working with CGA to manage their crops. They are now more aware of exotic leaf miners, their parasitoids, and the information available on managing them but probably will not drastically alter their practices unless, it becomes a major problem. This community type event proved to be an excellent way of engaging with many farms and industry support people.

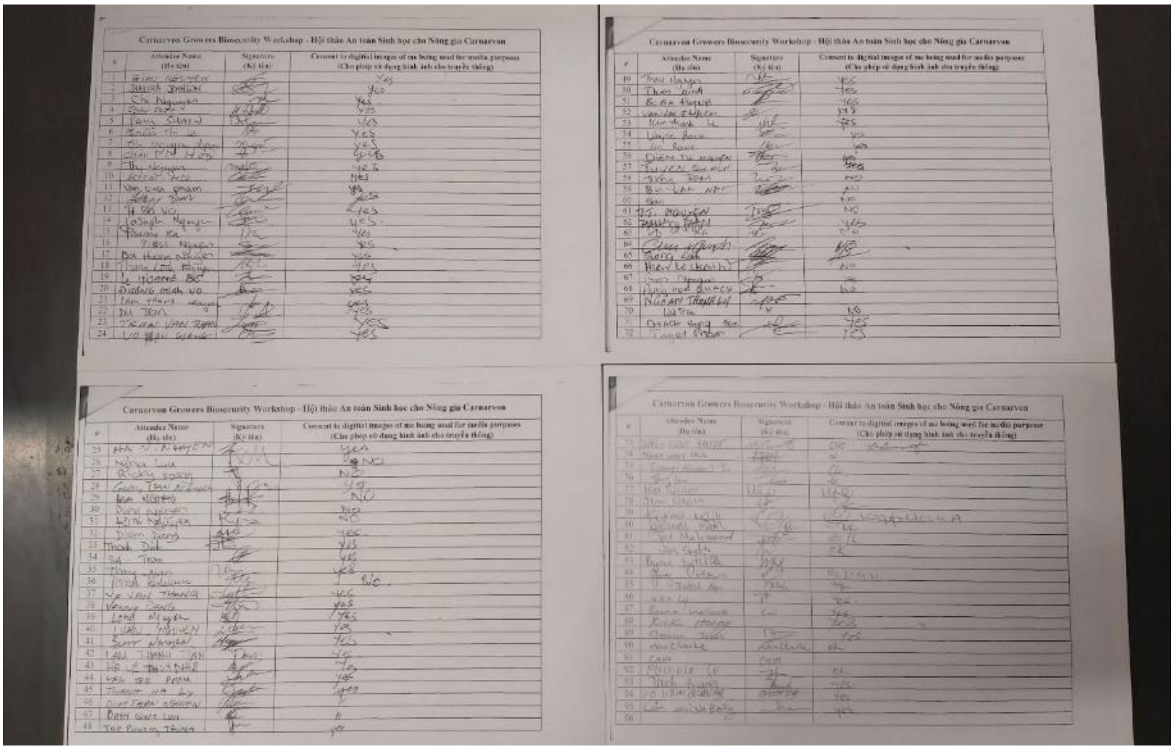
19 Carnarvon Workshop growers and industry participants at Mr Tham's shed

20 Project leader showing parasitoids with microscope video camera

21 Local DPIRD officer providing a local perspective on pest and disease management

22 AUSVEG presenting, Vegetables WA translating

23 Two essential workshop tools



24 There were 95 participants on the attendance sheets representing approximately 40 farms as well as industry support people

Broome Farm Visits

Broome has a small number of commercial horticulture farmers and was included in the workplan. On the advice of the local DPIRD horticulture team it was planned as a series of individual farm visits. The region has recorded ASLM, and the project team found numerous mines in gooseberry weed on old mango orchards and on dwarf snake beans in the TAFE Horticulture facility in the town. The region was also hit hard with the initial incursion spike of FAW. Severe damage was recorded on corn, maize, forage sorghum and Rhodes grass irrigated pastures but, that pest pressure is now much reduced. DPIRD has surveyed the region for ASLM parasitoids and has results for the beneficials.

Despite these two exotic insect incursions, the biggest problem facing commercial growers in the area is Cluster Caterpillar. After discussion with the farm managers, it was obvious that this was the result of over spraying broad-spectrum insecticides on advice from southern agronomists. This was the same problem that forced NT Vegetable growers to adopt more sustainable and successful IPM practices. Any advice on ASLM and FAW needed to consider that an IPM program for the exotic pests was going to fail in this toxic environment. The local managers understood this but were not empowered to change the pest management strategies. The DPIRD officers offered to begin a trial of boosting local beneficials at the research plot at Roebuck Bay between two of the larger farms.

Closer to town a smaller mixed vegetables and mango grower was implementing a more sustainable system with varied success. The team found Cluster caterpillars and the Spined predatory shield bug nymphs in his Asian Basil and while there were leaf mines on the gooseberry weeds, they were not evident on the almost finished snake bean trellises. These two observations point to natural parasitoids and beneficials in the area having some control on these pests. At the TAFE plot in town there was significant old mines on the dwarf snake bean, but production was not severely affected, and the TAFE staff were very keen to teach the IPM principles and practices to their students. They were eager to keep copies of the leaf miner management and monitoring guides.

Key Learnings: The project information provided strong support for TAFE to continue teaching IPM principles and practices and linked them to the AUSVEG website for more information. Evidence was seen in the area that parasitoids and beneficials are present in the area and can provide a measure of control when used in a coordinated strategy using softer and more specific chemical options. The team provided local managers with information to inform their owners and agronomists of the impact of counterproductive insect management programs and the local DPIRD is looking to provide support for IPM practices.

25 Discussing Leaf miner Management Guide at Broome TAFE

26 Inspecting snake beans in Broome

Kununurra Workshop

A formal workshop was held at the DPIRD Frank Wise Institute Kununurra, on 19/10/2023. Kununurra, like Darwin, had been strongly impacted by ASLM and FAW. One farm in the Packsaddle area had lost a crop of lettuce grown for the local market to ASLM and recorded damage on other vegetable and melon crops.

Local melon producers reported an increase in leaf disease which on closer inspection was secondary infection from ASLM damage. This incursion followed the FAW incursion which was damaging on the major maize field crop in the area. They were learning to manage crops using IPM strategies that included planting times and selective chemical applications.

The workshop was well supported by the DPIRD project team and local staff, agronomists from Ord Co and project team members from NT DITT, NT Farmers VegNET and AUSVEG. The workshop followed the proven formal format used in Darwin, Ayr, and Bowen workshops. Major growers participated, as well as agronomists and support staff from DPIRD, Ord Co and North Australian Cropping Research Alliance (NACRA).

The discussions that occurred during and after the presentations and practical session were much more “This is what we see happening in our crops” rather than “What if”. The key observation on both exotic insects ASLM and FAW was that the farmers were coping much better as time went on. They were observing the insect pressure had come right off even though the pests were still present. This indicates their IPM management strategies were beginning to work well. There was total agreement that the research results presented on parasitoids and beneficials matched what they were seeing in the field. Growers were keen to understand how they could monitor for parasitoids in their crops.

These growers are impacted by WA plant movement restrictions out of the Kimberley for ASLM which is a constant reminder of the value and impact of biosecurity on their enterprises. The permits table was seen as reinforcement of their current chemical management practices. The diagnostic developments were seen as interesting, but like Bowen growers, was seen as more suitable for biosecurity surveillance and monitoring spread rather than managing crops. The resistance information was well understood and matched their infield experience as well.

Key Learnings: Kununurra growers are using IPM strategies to manage ASLM and FAW. There is no need for practice change just some fine tuning as more information on resistance and local parasitoids becomes available. The experience in Kununurra provides confirmation of the management strategies recommended by MT 20005 and MT16004.

27 Kununurra Workshop

28 Practical session Kununurra

Gatton Smart Farms Agri-tech Field Day

Visitors to the Smart Farms Agri-tech Field Day in Gatton had the opportunity to look at a preferred host trial of common vegetable crops in the Lockyer valley. Plants that had been mined were tagged to show the damaged leaves. The project had a display of extension materials and microscopes set up to view the leaf miners and their parasitoids. Information was also provided on FAW and its parasitoids. Presentations stressed the importance of IPM programs to deal with both these newly established pests and to build resilience in farming systems. The IPM systems for biosecurity resilience focus, was the innovation focus for the project display. Approximately 600 people moved through the exhibits.

29 SLM crop preference trial and presentation

30 Display at the SLM & FAW information site

Bundaberg Farm Visits

The team visited two major chilli producers in the Bundaberg area and discussed the threat of leaf miner to their businesses. One of the growers also has a role in providing transport and agronomic services to growers from Bundaberg to Townsville, particularly to Vietnamese growers. This grower was very interested in the material shown to him and requested a Vietnamese version as quickly as possible. Vietnamese growers are becoming more common in Bundaberg and are well established in the Upper Haughton region of the Burdekin and Alligator Creek region of Townsville. FAW was found in the forage sorghum volunteer plants left over from the green manure crop but was not causing the grower any concern. This would indicate a level of natural control by beneficial insects probably from the Burnett River corridor near to the chilli planting.

The other chilli grower has been moving to a sustainable pest management strategy for his enterprises for some time. He wholeheartedly supported the move to more IPM based systems to provide more sustainable outcomes for growers into the future. The business has diversified into many processed products to maximise the use of all products grown and provide a more diverse income stream and market window.

The third grower visited works on an organic fruit and vegetable farm and a hydroponic fruit enterprise. He has recently come from a major capsicum producer in the Gumlu area. He had some insights into movement of the first instar of FAW onto and into the capsicum fruit from outside the capsicum planting. Monitoring of egg masses showed no eggs on the capsicum plants, but much of the fruit was penetrated and unsaleable with FAW caterpillars throughout. This challenged the accepted biology in the area, until a scientific paper showing FAW first instar can indeed “parachute” into a crop on a fine thread of silk was uncovered by personal research. The grower was disappointed in the early dismissal of this theory by the local department and urges research and extension officers to keep an open mind and not dismiss observations that don’t fit accepted theories. The implications for managing exotic leaf miner flies are clear. Each region must validate the existing theory or update it with rigorous observation and monitoring.

Key Learnings: The Bundaberg growers visited had a good understanding of IPM systems leading to improved sustainability. They are well positioned with agronomic experience and can access a variety of support services to implement changes to their growing systems as required. Furthermore, they have the experience to research and trial their own solutions to agronomic and economic challenges.

31 Chilli paddock Bundaberg

32 Chilli packing line Bundaberg

Bundaberg Workshop

The Bundaberg workshop was held on 8/11/ 2023. It was added to the program at the request of the Bundaberg Fruit & Vegetable Growers (BFVG) based VegNET officer. It could easily be delivered as the workshop team were all in SEQ for the Smart Farms field day at Gatton and the workshop materials were all available. This followed very favourable reports from the FNQ workshops and requests from local agronomists who were seeing symptoms that were consistent with SLM damage. The 18 attendees were all either agronomists or worked in industry support.

The agronomists engaged throughout the presentation and practical components of the workshop with inciteful questions and comments that demonstrated an in depth understanding of pest management in complex growing systems. This was reflected in the final discussion and considered answers to M & E questions.

Bundaberg was one of the few growing areas that did not have a specific Preparedness Plan produced in project MT16004 but did have access to the prepared information through previous multi-topic workshops and newsletter articles from BFVG. Despite this, and the fact that some of the agronomists there have been in the district for many years, the awareness of the extension material from MT16004 was limited to a few agronomists that had accessed the MT16004 website.

Key Learnings: The Bundaberg workshop and farm visits highlighted the power of VegNET when it is working well. The local VegNET officer responded to the enquiries from local agronomists about the workshop series, as they had heard about these through their company networks following the FNQ workshops. They were aware that the leaf miners were present in the next major vegetable growing region of the Lockyer Valley where seedlings were regularly sourced for the Bundaberg region.

The VegNET officer then worked with the project team to arrange time, venue, and attendance for a very successful workshop. A great example of what VegNET was set up to do and a fulfillment of the vision of the early pioneers in the VegNET programs. It is powerful as a clear example of VegNET "working well".

This workshop also reinforced the value of targeting the agronomists in a region. These are the "advice givers" and have the time, experience, and knowledge to incorporate ideas into the local growing systems and can see the wider agronomic and economic impacts of new incursions and the proposed management strategies. Having this group aware of the signs of an incursion, the damage and possible crop outcomes ensures that the growing community is prewarned across the region.

33 Presentation at Bundaberg Workshop

34 Bundaberg practical discussions

Summary Key Learnings: Geraldton to Bundaberg.

There is a clear distinction between regions in the North where ASLM has been detected and those where the exotic leaf miner flies are yet to occur. The experience of those regions with the ASLM in NT and WA is that after an initial incursion peak the pressure is reduced and requires minimal chemical management. This is the same experience with FAW in most areas. Initially there was extensive crop damage and a concentrated diverse chemical attack that proved of little value. Growers have found ways to manage both pests and are reporting reduced pressure over time by implementing an IPM approach.

IPM is central to the management of exotic leaf miner species but cannot operate in isolation of other insect management strategies in a farming system. Building on-farm resilience through IPM systems will be critical in managing further incursions that will likely arrive with a similar large suite of chemical resistance.

This has been reinforced with the current findings of active parasitoids and predators on native Spodoptera sp., which have been reducing the impact of FAW in the same regions where ASLM has been recorded. This concept is backed up by the recent arrival of Papaya mealy bug into Darwin which thankfully arrived with its most effective parasitoid.

There is a range of knowledge and effective implementation of IPM systems across the different farming regions and farming communities in Northern Australia. Sophisticated IPM systems on large corporate farms with imbedded agronomy, along with smaller farms with good agronomic support, either from resellers or local departments, are proving effective. Research and development of techniques, such as infield diagnostics and genetic resistance monitoring, need to feed into existing IPM strategies, and a new pest incursion then becomes a fine tune or tweak to current IPM programs.

Established growing regions that are well served with agronomic support from experienced agronomists and reseller companies with sustainable objectives were very receptive to the concept of building resilience within a growing system using all the tools available to them. The interaction with the project team was inciteful and constructive. There was an atmosphere of professional cooperation in discussions and suggestions.

Other communities through language, lack of access to information or isolation are only beginning to realise they need to transition to a more sustainable pest management system. This poses a clear danger as a pathway for further incursions to establish and should be a priority for continued engagement, upskilling, and surveillance. These communities need to build a relationship and trust with an on-going support person. VegNET officers are an excellent example of a long-term project framework that can build these relationships and provide the support need for these groups to start their practice change journey.

VegNET was seen to play a key coordination role in these communities in bringing the most experienced and influential advice providers together on “neutral” ground to learn, discuss, and plan. VegNET officers who know and understand their regions were seen to have built trusting relationships that enhanced the value of the project and workshop series. There is an urgent need to clarify the role of the regional VegNET officers interacting with major vegetable R&D projects and clear expectations and contact pathways identified within these major projects. The interaction with VegNET RDO’s ranged from “I don’t know anything about it, I will put your flyer in the newsletter”; to making contact with the project team, inviting them to do a workshop and helping with the logistics and attendance. Over $1M of growers levy funds were invested in the leaf miner project across all States and Territories. There is a clear statement in the project documents that references VegNET as part of the extension pathways. Major vegetable projects like this should be on their radar and in all their workplans.

Building resilience in our farming systems through sustainable pest management strategies is critical to our response to further pest incursions. This includes taking advantage of our native biodiversity of parasitoids and beneficials as well as targeted release of commercial beneficials. Along with biological pesticides and targeted chemical use with a clear understanding of off target impacts.

Investing in ongoing extension activities in IPM systems is needed and should be imbedded in programs such as VegNET and other horticulture industry extension and engagement programs. Funding bodies often remark this has all be done before but there are always new growers and new communities of growers, such as migrants from sub-Sahara Africa we are now seeing in Northern Australia. Even existing established IPM systems face changing pressures and there are new tools and methodologies being released constantly that need to be incorporated.

Workshop Running Sheet example.

Running sheet Bowen Workshop 6 July 2023

Time	Topic	Who	Activity	Resources
2.00	Welcome & House keeping	John D, Greg O		
2.10	Intro		Round the room	
2.20	Biosecurity risk pathways	Shakira	Presentation	Powerpoint
2.30	Fall Armyworm update	Subra	Presentation	Powerpoint
2.40	Leaf Miners Life cycle Hosts and Monitoring and diagnostics	John & Praise	Presentation	Powerpoint
3.00			Practical	Microscopes, samples, video, Genie LAMP
3.30	Parasitoids	Peter R	Presentation	Powerpoint
3.40			Practical	Microscopes and specimens
4.00	Insecticides trials and resistance	John	Presentation	Powerpoint
4.20	Leaf Miner extension materials	Greg	Show and ask questionnaire.	Leaf miner management and monitoring guides
4.30	Discussion on local experiences	Greg	Discussion	
4.45	M&E- worksheet questions	Greg	Discussion	M&E workshop Discussion questions
5.00	Close WS	All		
	BBQ & refreshments	Greg	informal	Drinks and food
6.00pm	Finish			

Full Workshop Team

Not all team members were at each workshop, depending on location and availability.

John Duff	Q DAF
Praise Frezel	Q DAF
Subra	Q DAF
Brian Thistleton	NTDITT
Peter Ridland	Uni Melbourne
Duong Nguyen	NSW DPI
Shakira Johnson	AUSVEG
Greg Owens	AUSVEG - Extension Consultant
Helen Spafford	DPIRD
Penny Goldsmith	Ord Co

Appendix 4.2

Grower and Industry M&E Survey Responses

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by Greg Owens

MT20005
Exotic Leaf miners
Grower and Industry M & E Survey Responses

There were 84 growers and 60 industry support attendees at the extension events held between May and November 2023 from which responses were collected. Growers included all growers attending the workshops or who answered the questions as informal interviews during farm visits. The industry support group included everyone else connected to vegetable and fruit production in workshops or meetings that answered the survey questions either written or as an interview. This included agronomists, suppliers, extension officers, development officers, biosecurity officers and other departmental people, industry association representatives and supply chain members.

The survey questions targeted the awareness, reach and usefulness of the extension material produced in MT16004 as well as the intensions of attendees to use and disseminate the information already provided and being updated. The survey questions could contain multiple answers and the attendees were given the option of not answering if they preferred. The surveys were worded differently on some questions to reflect the different roles of growers and the industry support group in responding to pest incursions in the horticulture industry. The responses for the two groups for similar questions have been presented together to highlight similarities and differences between the two groups and show opportunities for more targeted extension efforts.

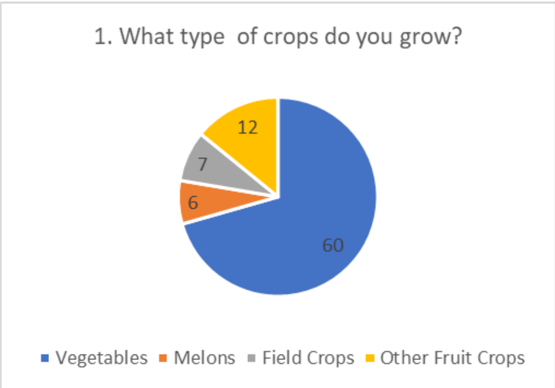
The questions gauging whether this was an appropriate use of growers' levy money to produce the materials and run the workshop series allows the project to evaluate the impact of these activities on these attendees. The responses to the biosecurity questions at the end of the survey reflect the knowledge of and the value growers and industry put on on-farm biosecurity to manage pest incursions.

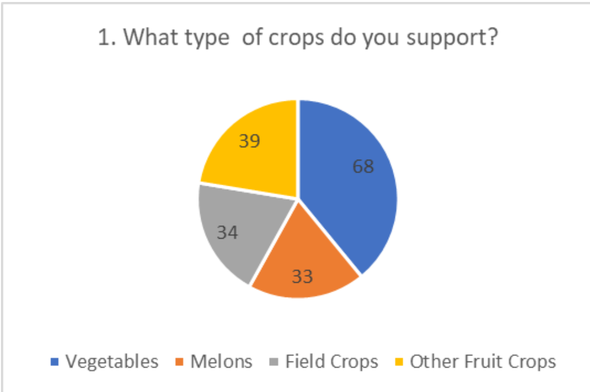
Growers

Industry Support

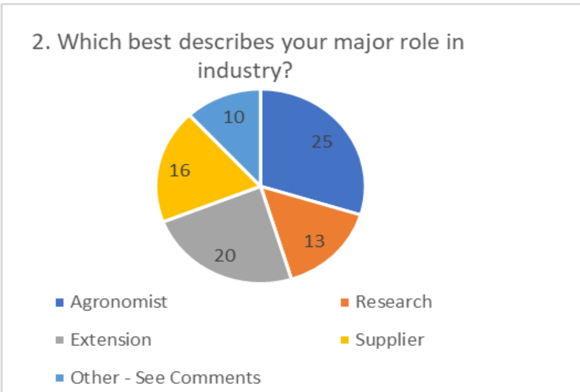
1. What type of crops do you grow?

……………support?



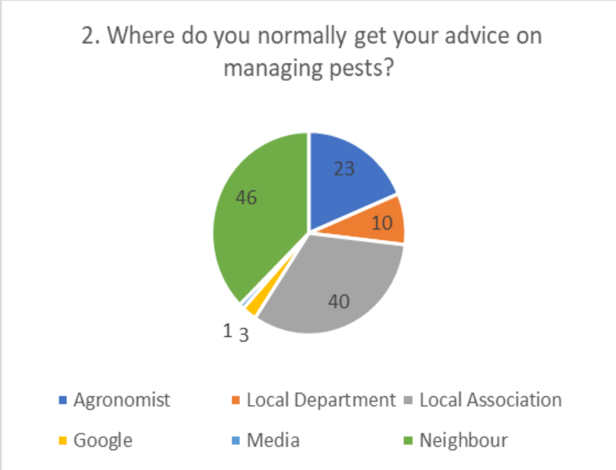


The grower attendees overwhelmingly identified as vegetable growers with some mixed farming enterprises. The industry support attendees dealt with a much wider set of horticulture crops and as would be expected mostly had a broader view of the topics discussed.



The industry support group identified themselves as 25 agronomists, 20 extensionists, 16 suppliers, 13 researchers and 10 others which included biosecurity officers. Some saw themselves in multiple roles such as a supplier and an agronomist, or research and extension.

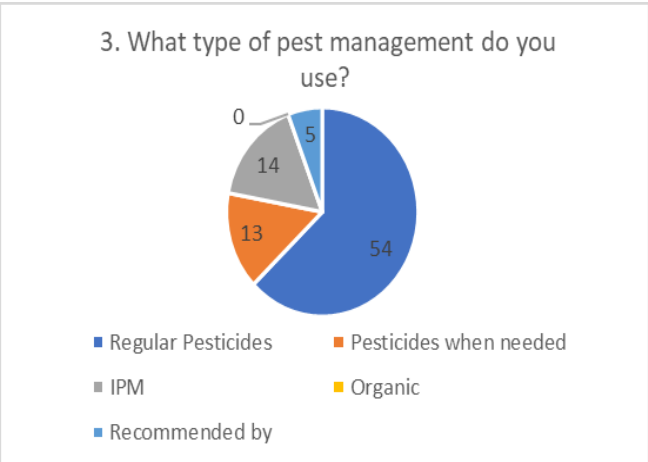
2. Where do you normally get your advice on managing pests? **……. information?**

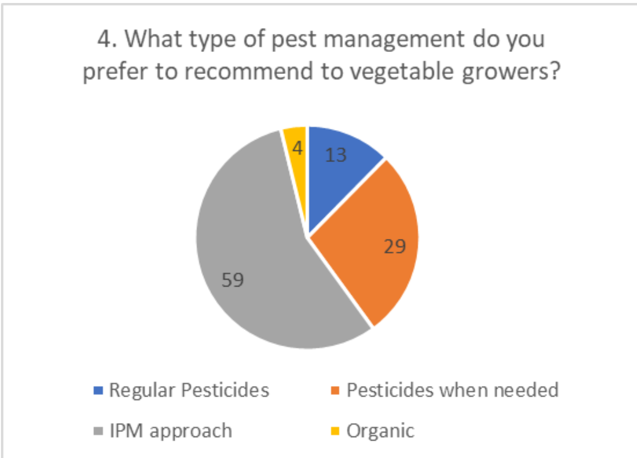


The growers' responses to this question were very location specific. In Carnarvon where there is a very active Growers Association and Vietnamese Community Association, advice came strongly from those associations. In more established growing regions with agronomic support, agronomists rated highly. In all regions a trusted source of information for them was their neighbors. The local departmental officers, the media and internet were not noted strongly as a source of trusted information.

The industry support attendees had a more even spread of information sources including field trial data conducted either by departments or within their companies' activities. Engagement with the local department is also higher in this group and scientific publications also being cited as a common source of information. This suggests that they should be the major target for more complex management guides and the associated extension effort.

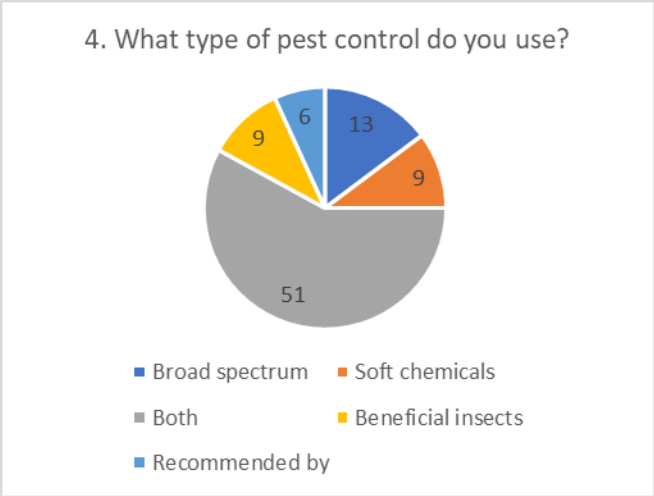
3. What type of pest management do you use? **…………recommend?**

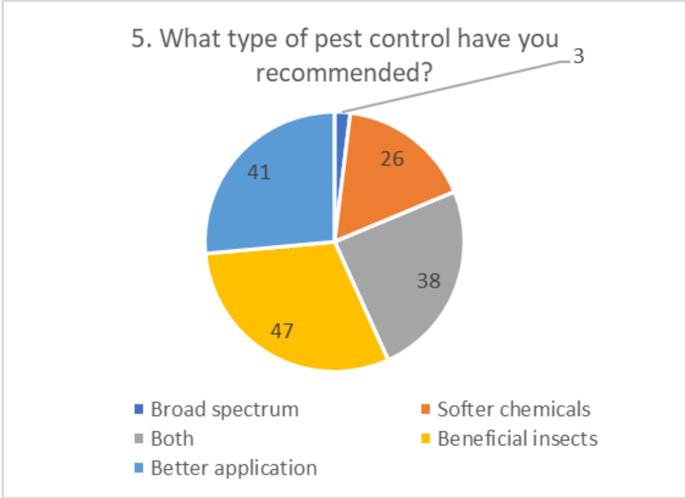




There is a clear difference between the growers' actions and the advice they are receiving. This could be because of several factors. There was a large influence of growers from NESB backgrounds in the survey results that have not had strong support to move to a more IPM approach which is seen as a risk by growers and so they revert to regular spray patterns. It may be that agronomists giving the IPM advice are from more established regions where contact with this extension program was higher for this cohort. It may be the perceived cost of soft chemicals, getting permits and reliable beneficial insects also contribute to this outcome.

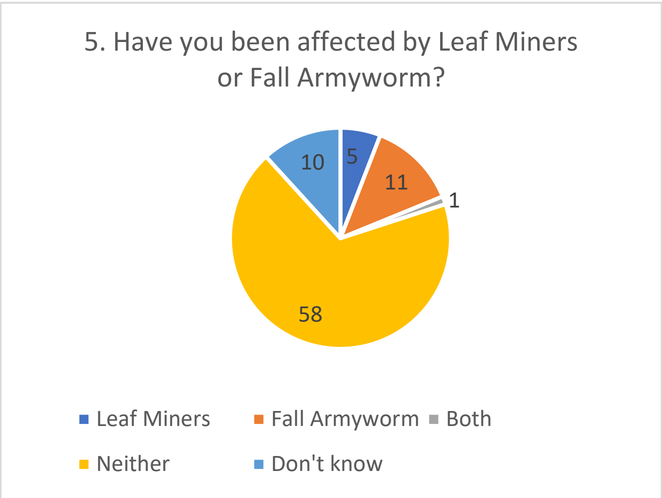
4. What type of pest control do you use? ....have you recommended?

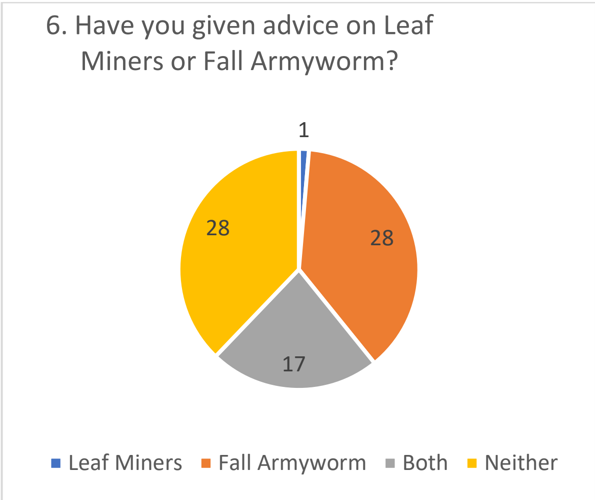




There was a very similar response to this question. The growers and agronomists are using what chemical tools are available both soft and broad spectrum. The agronomists are more likely to recommend a variety of tools to the growers. This can be seen by the much higher recommendation of beneficial insects, but this is not matched by the growers' use of beneficial insects.

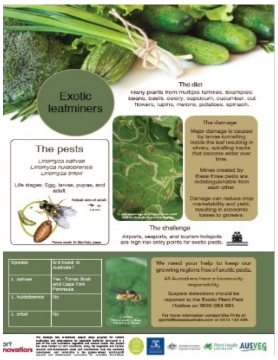
5. Have you been affected by leaf miners or Fall Armyworm? .... given advice on LM or FAW?

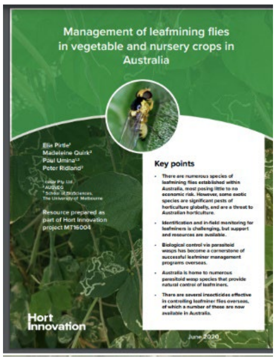


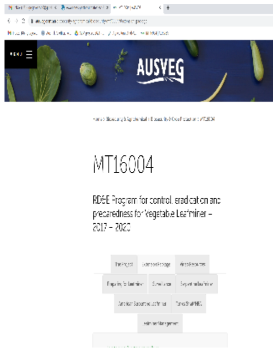


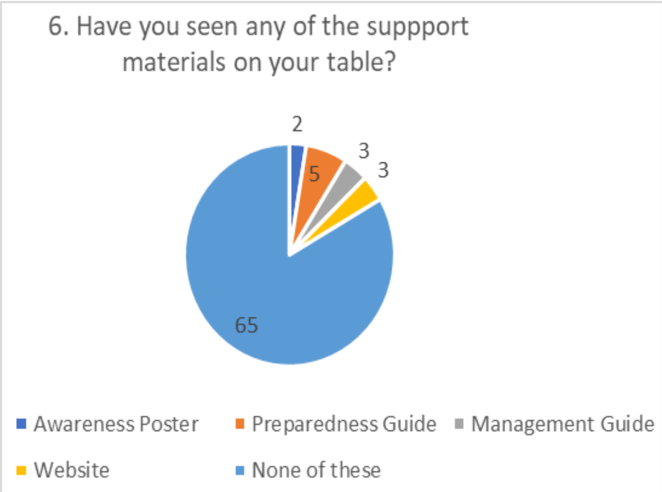
Only a small number of growers responded as having been affected by either exotic pest and only one grower from Kununurra with impacts from both. This is very different to the response from the industry support group where more than half had given advice on one or both pests. Again, the data may be impacted by the big group in Carnarvon where neither pest is yet present. It does show that the agronomists engaged by the project are aware of the exotic incursions and are giving their growers advice on their management. This again supports the concept of targeting this group, in particular, with pest management strategies for new and likely incursions.

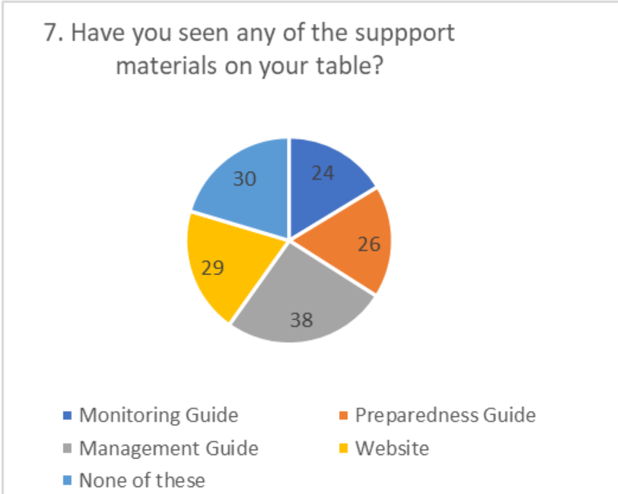
6. **Have you seen any of the support materials on your table? Tick the ones you have seen before today.**





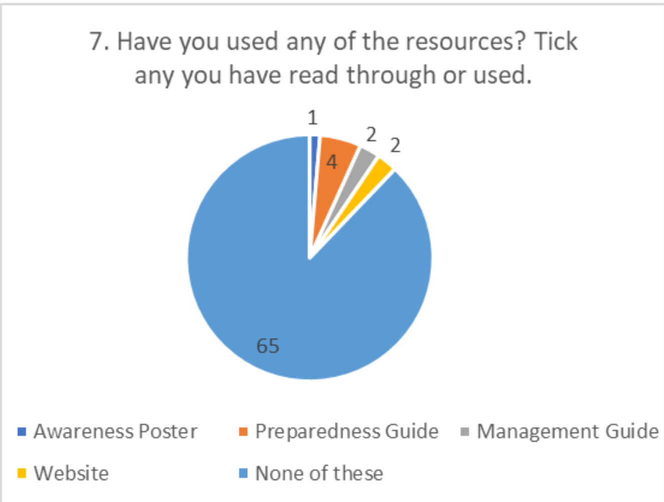


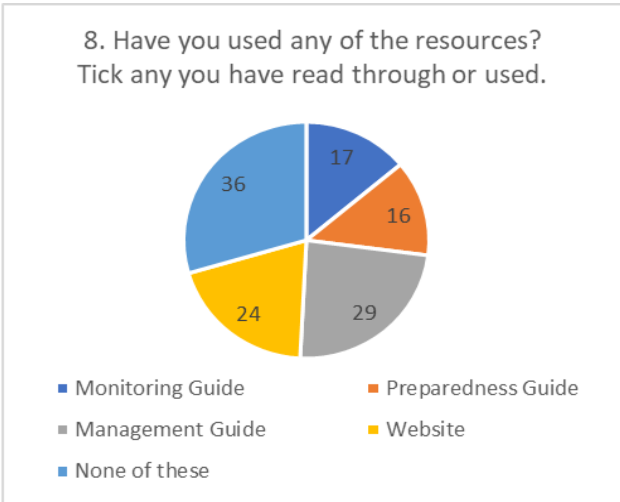




A very smal number of growers surveyed had previously seen the extesnion materials developed by MT16004. Around half the industry support group of 64 had seen all the information and the other 30 were not aware of this information. This provides a very real opportunity to target these key advisers with new pest managemt information.

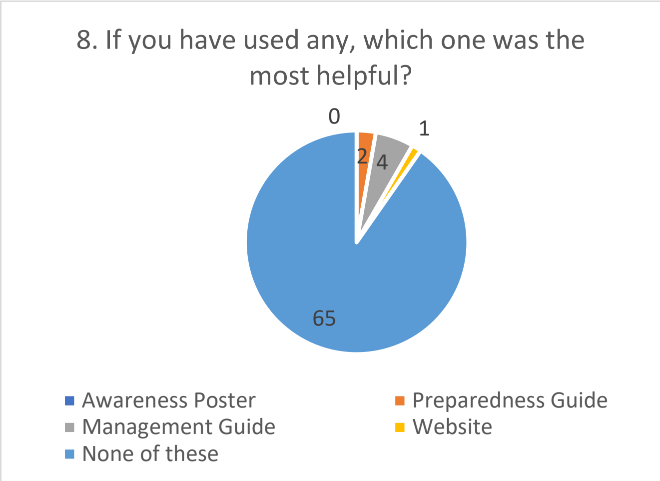
7. **Have you used any of the resources? Tick any you have read through or used.**

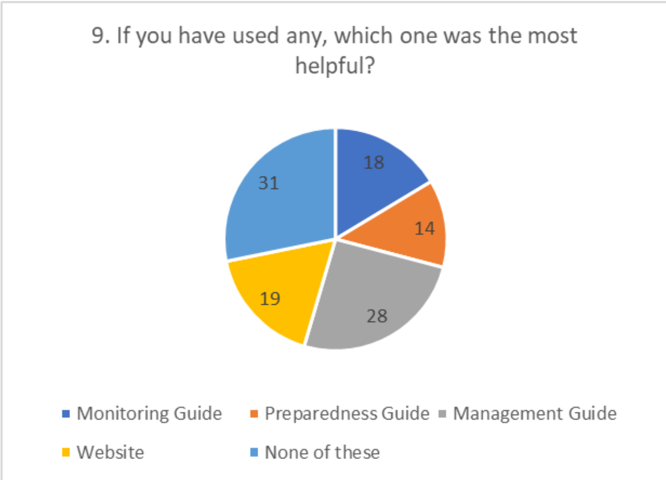




These responses mirror the previous questions responses for the growers. There is a noticeable reduction in the positive responses from the industry support group. Some of them were aware the documents existed but had not read through or used the information. This provides another opportunity to improve the interaction with this group.

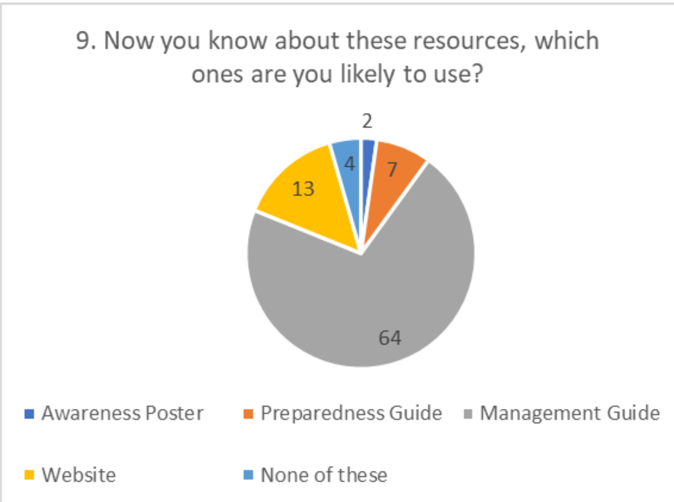
8. If you have used any, which one was the most helpful?

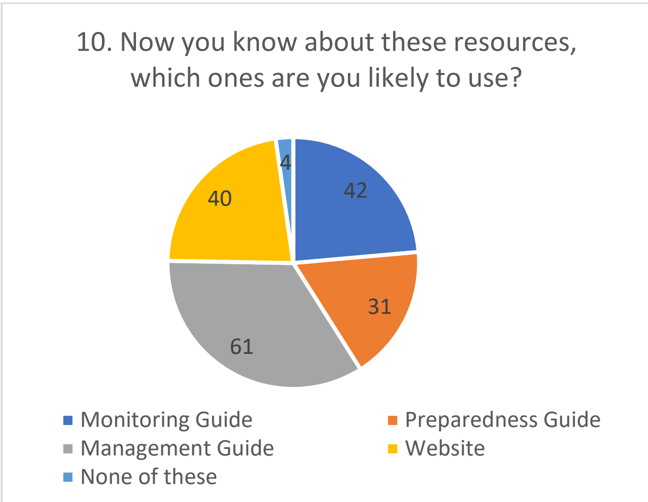




Of the resources seen by growers the management guide and preparedness guide were seen as the most helpful. The industry support group responses mirrored the previous question where all extension materials accessed were seen as useful.

9. Now you know about these resources, which ones are you likely to use?

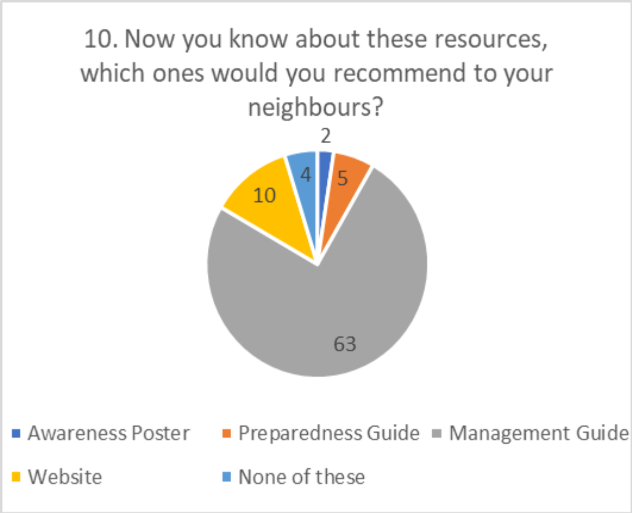


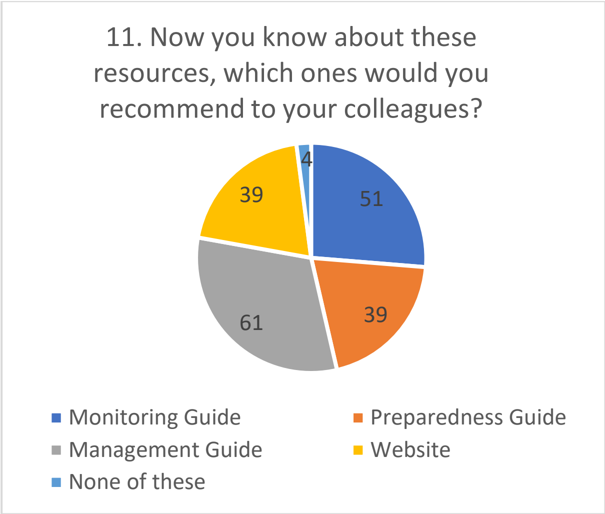


The Management guide was a clear favourite for the growers, with the website also getting favourable mention. They saw this document as providing useful information for them on the farm. This supports the production of a new set of grower guides for the exotic leaf miners for growers. The response from the industry support group showed their appetite for broader information regarding monitoring as well as management of these pests. The question clearly shows an aspiration (KASA) to use the materials, now that everyone who engaged in this extension effort knows what resources are available. As seen in the previous question though, it is most likely this increase in use will come form the industry support group.

10. Now you know about these resources, which ones would you recommend to

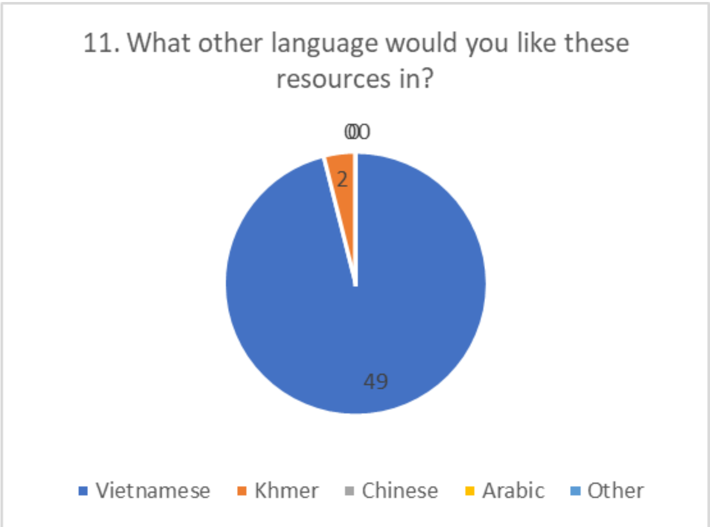
………… your neighbours? ………… your colleagues?





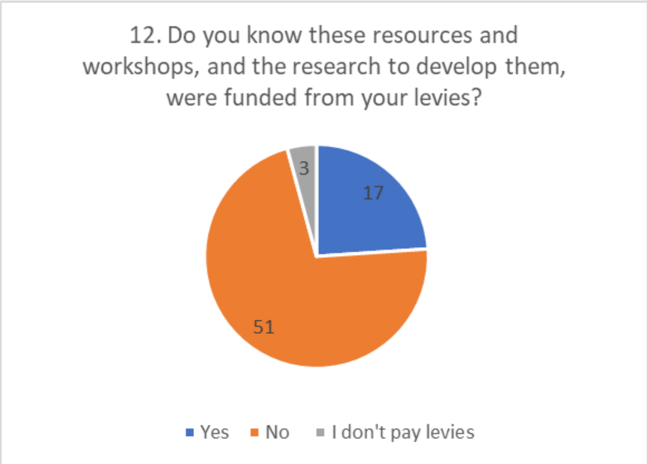
This question goes to the value growers and industry put on these documents. If they are good enough to share with neighbours and colleagues, then they are seen as valuable support materials from a trusted source. No-one would share documents that they have no confidence in.

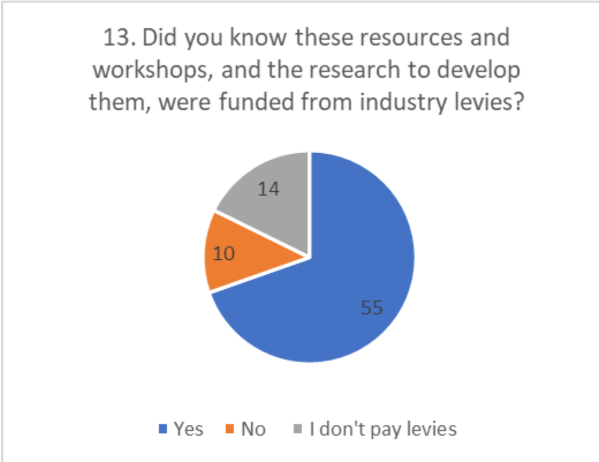
11. What other language would you like these resources in?



Vietnamese language versions are clearly the greatest need identified in this extension program. A small number for other languages identified reflects the current ethnic make up of the vegetable growing community in Northern Australia.

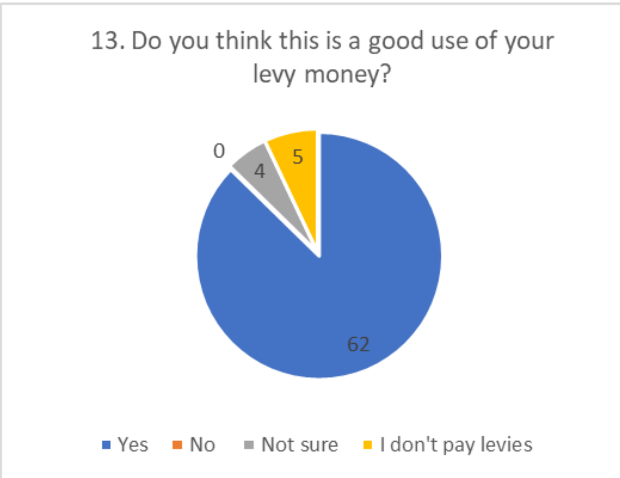
12. **Do you know these resources and workshops, and the research to develop them, were funded from your levies?**

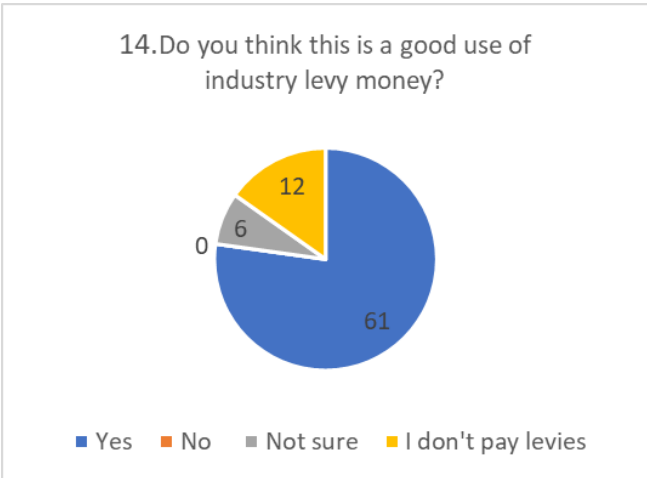




This question highlighted that growers still don't know how their national levies are invested. This question was further complicated in WA, as growers in Carnarvon pay a state and a federal vegetable levy. WA Geraldton growers mostly avoid the WA state levy by sending their produce to South Australia. It is incumbent on all levy funded projects to continue to ensure that growers are informed as often as possible on what their levies are providing. Industry players are much more aware of the role of grower levy money in R&D projects and programs.

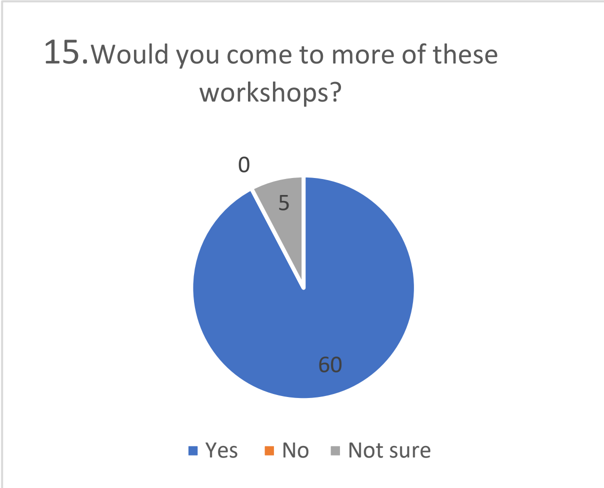
13. **Do you think this is a good use of your levy money?**





The growers overwhelmingly supported this project and its extension effort as a good use of their levy money. This is consistent with the response from growers about other projects when they are informed it has been funded by their levies. It is frustrating at times, but all project participants need to remember to continually remind growers of this. Industry knows how important this funding is and could also play a role in reminding growers where funding comes from for levy projects.

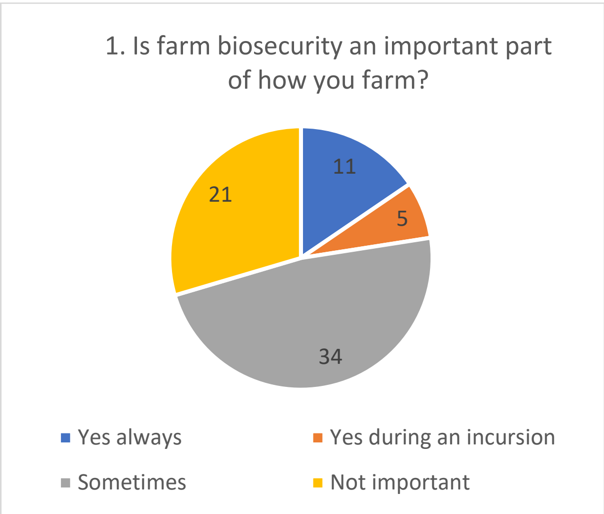
14. **Would you come to more of these workshops?**

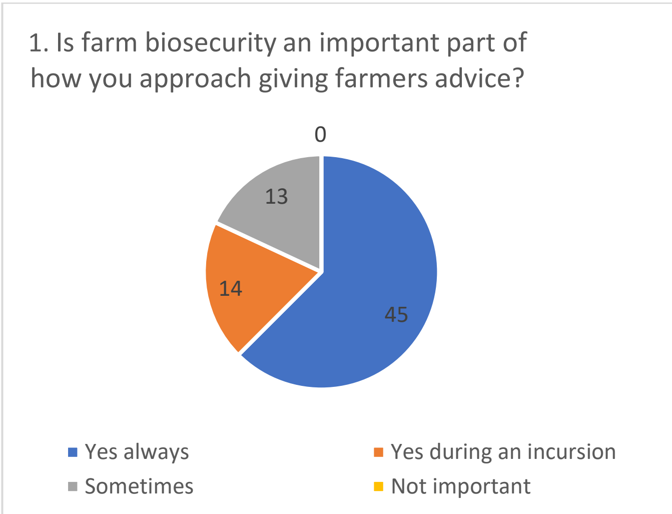


Almost everyone recognised the value in these types of workshops and would come again. The practical and discussion sessions allowed active participation and valued input from the participant and gave the project team the regional context for management of these pests.

Biosecurity

1. **Is farm biosecurity an important part of how you farm?** **…………. advice you give?**

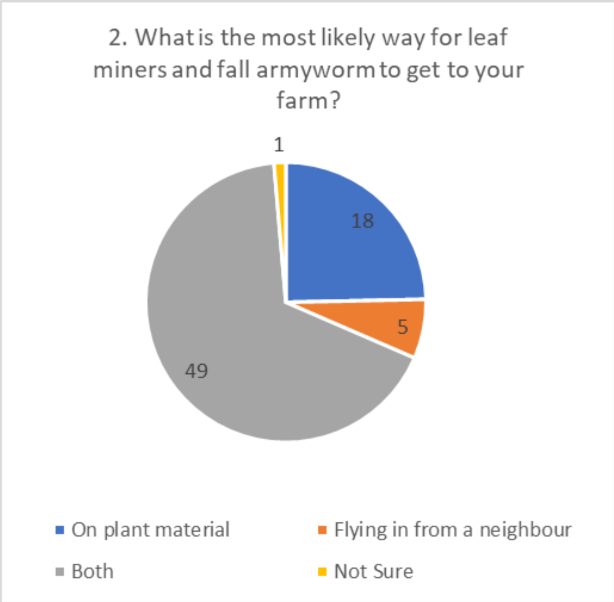


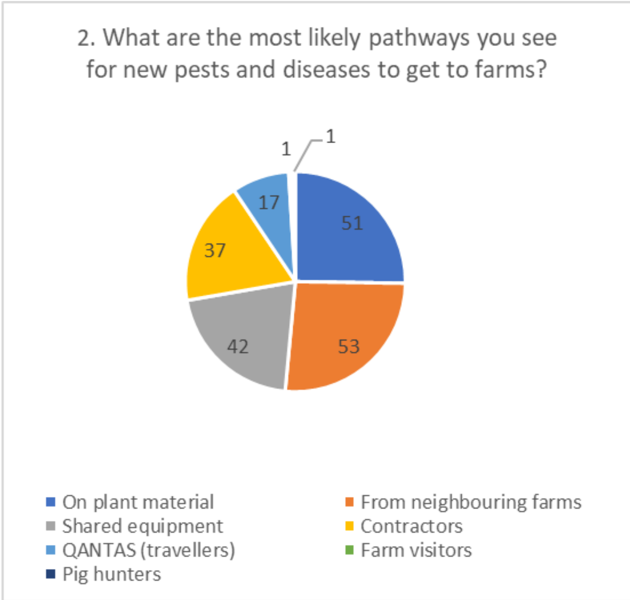


Only a small percentage of the growers have biosecurity as an important part of how they farm. The others obviously think about it "sometimes", but it is not a key part of their farm management. It can become important during an incursion. This project started its life as MT16004 as a preparedness project for a biosecurity threat and morphed into MT20005 when the exotic leaf miner flies had arrived in Australia. It does reflect the feeling among growers that these incursions are inevitable and there really is no financial or agronomic advantage in a detailed biosecurity plan.

The industry support group on the other hand, regularly gave advice on the importance of a farm biosecurity plan either always, sometimes or during an incursion. What may be lacking is advice on the financial benefits of an active biosecurity-based farming system that may encourage growers to develop these systems.

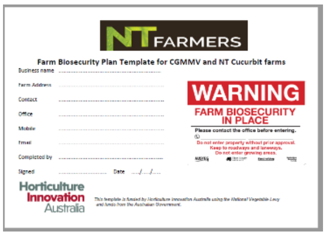
2. What is the most likely way for leaf miners and fall armyworm to get to your farm?

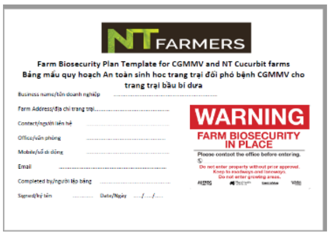


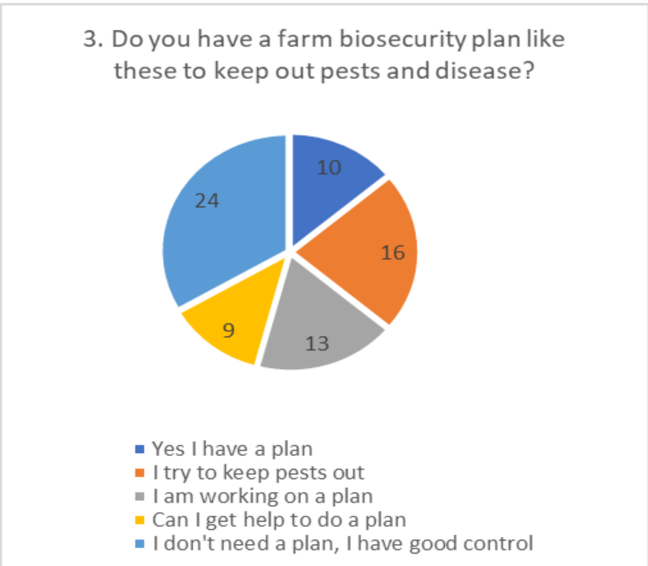


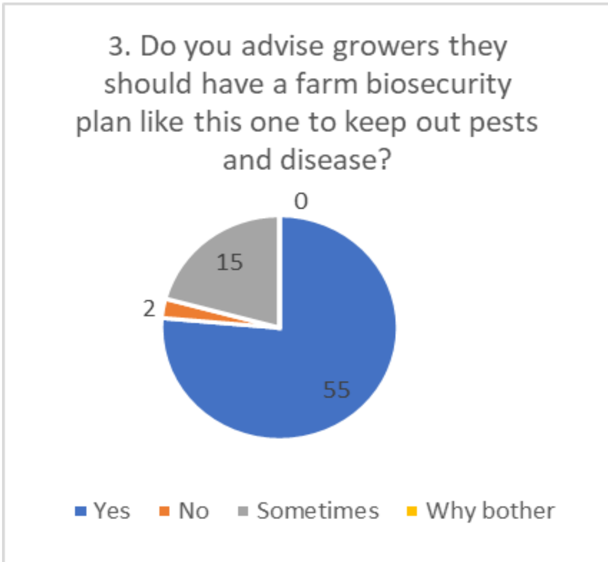
Most growers had a good understanding of where these introduced exotic pests could come from. The agronomists added a few more pathways to the original question and have selected multiple pathways in their responses.

3. Do you have a farm biosecurity plan like these to keep out pests and disease? ……. advise plans like these?









Again, the responses show a clear understanding of biosecurity as part of how to farm but much variation in its application as an on-farm biosecurity plan. Industry support is advising the growers to have a plan but maybe this needs more work to find ways around the barriers. Encouragingly 10 growers do have a plan and 13 growers are working on a plan. The fact that 9 responses were looking for help to develop a plan also provides opportunity to target these growers in the new industry biosecurity plan.

Analysis of Survey Question responses

The responses demonstrated that extension is not a one-off exercise. There needs to be ongoing interaction with growers and advisors in the horticulture industry. There was clear evidence that prior engagement with the topic and the extension materials allowed the project team to build on the previous knowledge and skills gained and had a strong impact on attitude and aspiration for continuous improvement in pest management (KASA). There was evidence of practice change on individual farms and a general level of commitment to start or consider advice from the extension materials in their management and advice.

The response about sharing the project resources with neighbours and colleagues shows that the information is valued and from a trusted source. The is a clear need in Northern Australia to have vegetable extension resources inlanguage especially Vietnamese. This will improve the effectiveness and the acceptance of this advice. There was almost uniform support for using levy money on this type of project, but it was confronting to see how many growers didn't realise this is the type of project their levy money was spent on. More work is needed here in all engagements with growers. Industry support personnel are much more aware of the funding models.

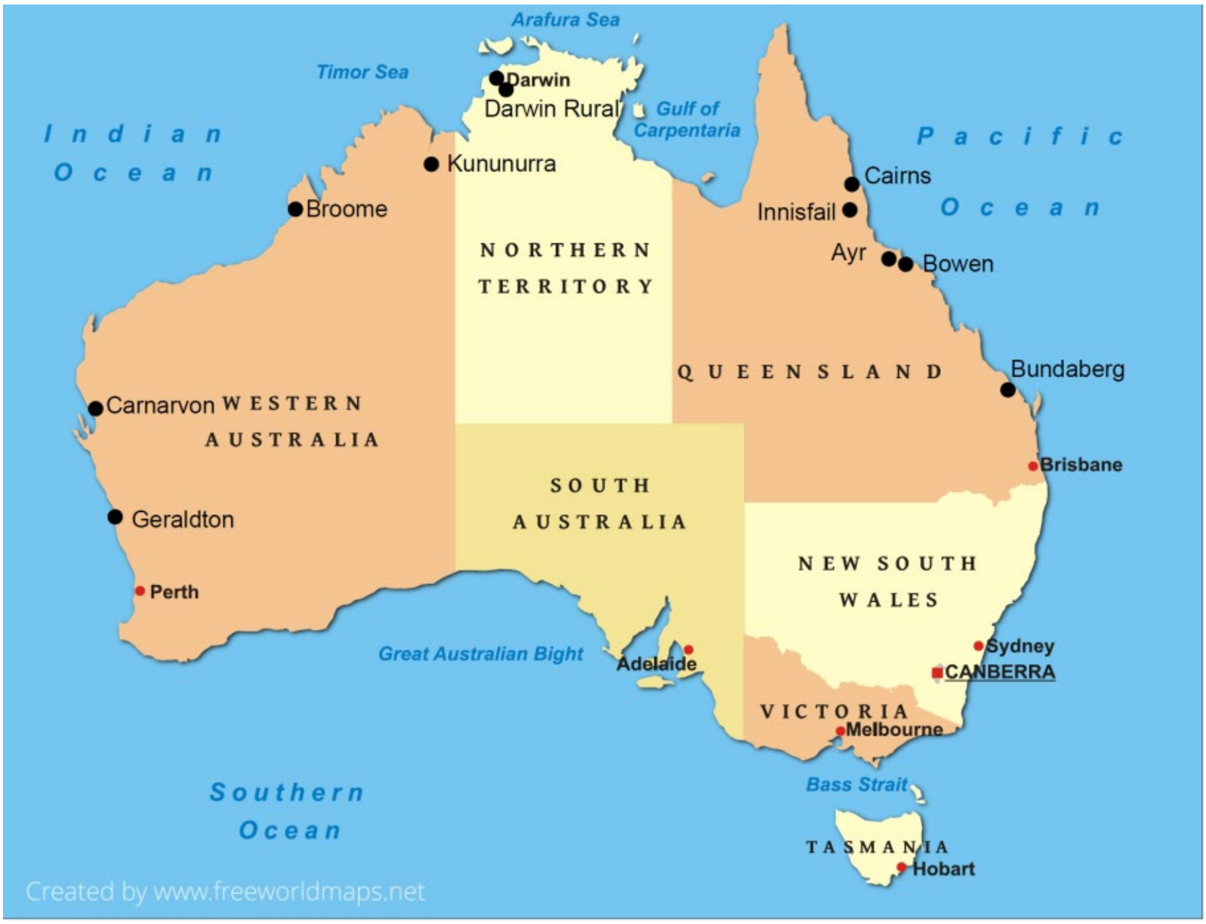
Biosecurity received almost universal support from the industry support people, a sound knowledge of incursion pathways and support for grower to have biosecurity plans. There are still growers that need to develop and /or implement on-farm biosecurity plans even though they have a reasonable grasp of incursion pathways. This is an area of ongoing need and requires a variety of engagement and support strategies and strong language support.

Appendix 4.3

Evaluation of Northern Australian Extension Program

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by Greg Owens



MT20005

Evaluation of Northern Australian extension program.

Where to from here?

The last activity in the Northern Australian leaf miner project workshops was a series of five discussion questions given to each table to discuss and note their responses to the statements/questions. In larger groups and where English was not the primary language, a whole group discussion was conducted, and notes taken on the group's contributions. During farm visits, the growers were asked the same questions in a rich interview format and notes were recorded on their thoughts to some or all the questions.

A review of the responses and any recommendations arising from the questions is summarized at the start of this document. The discussion worksheet and all the responses to each of the questions from each activity are provided later in the report. This review also correlates the responses to the discussion questions with the survey responses from earlier in the workshop to assess the consistency of the responses and highlight any increases in knowledge or changes in intention as a result of the extension effort.

1. **Leaf Miner grower guides being updated and republished for vegetables, melons, onions, and potatoes (separate levies)**
   Are they useful and informative or too complicated, hard to find and a waste of time.

   Review of responses
   The grower guides are seen as useful and valuable at all activities. The guides were seen as informative and had enough information for attendees to understand the key messages. The graphics were commented on favorably and "please add" more pictures and produce related videos commonly referenced. This correlates well with the answers to survey questions 8, 9 & 10. There was strong agreement with survey question 13 that this was a good use of levy money.

   There was some comment that growers and agronomists did not know the resources existed or where to find them. This again correlates with survey questions 7 & 8. It was noticeable that this response was more common in activities that were the first contact from the project. Regions that had attendees from previous leaf miner workshops maintained a legacy of knowledge that could be built on by the project.

   There is a strong call for these resources to be in Vietnamese which reflects the ethnic make up of vegetable production in Northern Australian. This also correlates strongly with the answers to survey question 11 responses from growers and industry support where Vietnamese versions were requested at the ratio of 77:12 for other languages.

   Recommendations arising
   - An updated version of the Grower or Management Guides has strong support to be produced, which gives solid backing to what is happening currently.
   - Any new version of the Grower or Management Guides should be produced in Vietnamese as well as English for Northern Australia.
   - An active (not passive) engagement strategy needs to be maintained in Northern Australia to ensure growers and industry are aware of newly released extension materials.
   - Agronomists, extension officers and other advisors are a key target for ongoing engagement and systems development.

2. **Rapid accurate diagnostic options now available in local departments and potentially to industry.**
   Do the techniques shown at the workshops offer better options for management?

Review of responses

The responses to this question did not display overwhelming enthusiasm for these techniques. The most common comment was that it would need to be done by someone else, such as Primary Industries Departments, or commercial laboratories. The techniques were seen as very useful for biosecurity surveillance and identification of new pests in the growing regions. Cost was seen as a barrier for smaller growers and access from remote regions to the technology was seen as a problem.

Most agronomists are already managing the pests by monitoring the impact on their crops in the field and reacting to damage using appropriate IPM strategies. They saw the value of these techniques more for confirmation of their management and for research on new incursions.

These responses clearly show many of the attendees have gained and understood new knowledge (Bennetts level 4 KASA) and have aspirations (KASA) to incorporate the results of the diagnostic tests in their management when they become easily accessed and affordable. There was a commitment to practice change in their attitudes (KASA) but the time is not quite right for practice change (Bennetts level 5) at the farm level.

Recommendations arising

- Regional departmental officers are supported to have access to the rapid diagnostic techniques either in-house or through existing networks.
- Commercial laboratories are approached to investigate if they can provide a timely, cost-effective diagnostic service to industry.

3. **Does the requirement to protect the parasitoids for leaf miners and for FAW fit with the other pest management requirements on the local vegetable and other affected farms? Can we make it work?**

Review of responses

There was a range of understanding (knowledge - KASA) of how IPM was required to manage the exotic leaf miners and FAW following the workshops. There was general acceptance that a chemical only approach was not the answer and that IPM offered a more sustainable outcome for the farming systems in the North. A good percentage of the farms and almost all agronomists and advisors (Survey questions 3, 4 &5) were already recommending, using, or starting to implement IPM practices. This was strongly influenced by the sophistication of the farming practices in each region and the range of agronomic support available.

Impediments to implementing IPM quoted such as can't have live insects in retail produce, even beneficials, spraying for lots of other pests, cost, and availability of beneficials and actions of nearby farms. A few points raised suggest that there is more work to be done on the basic biology of pest behaviour. There was an increase in knowledge of beneficial insects and parasitoids that impact exotic leaf miner flies and FAW. It was yet to be seen in some regions without exotic miners whether the IPM systems currently in use will work when the exotic pest arrive. IPM was seen as a way of building resilience in a farming system to cope with new pests and diseases.

Recommendations arising

- IPM system recommendations for regions and crops need to be continuously updated and informed with new knowledge and incorporate on-ground knowledge of farmers and industry advisors.
- The vegetable industry, in conjunction with other horticultural industries could establish an on-going series of workshops to establish, then monitor, evaluate, and fine tune IPM systems recommendations in all regions, utilizing the VegNET network.

4. **Does the table of current permits for leaf miners offer alternatives for management? Will it be useful when deciding a response to these pests?**

   Review of Responses
   The permits table was gratefully accepted by almost all attendees. Responses demonstrated a high level of understanding (KASA) of the format of the table that highlighted the potential impact on beneficial insects and parasitoids for each of the permitted chemicals for exotic leaf miners. There were also several responses that clearly demonstrated growers and industry understanding (KASA) of the legal issues of chemical applications to crops under the APVMA regulations.
   While not explicit in the responses, there was general appreciation in the workshops that the gaining and maintaining of these permits was a good investment of grower's levy money which matches the response to survey question 13. (Attitude KASA) It was also clear from the responses that growers and advisors would incorporate the permitted chemicals in their IPM response to any exotic leaf miner incursion if appropriate. (Practice change)

   Recommendations arising

   - The permits table should be incorporated into the updated Grower Guides.
   - The permits need to be assessed against their effectiveness on exotic leaf miners and renewed as appropriate.
   - Permits need to support ongoing IPM systems.

5. **"We are not going to worry about it until it gets here. There is too much else to do." What is your response to this very understandable statement.**

   Review of Responses
   This was a two-part question and goes to growers and industry understanding of the value of having a biosecurity plan in place before a pest or disease arrives. The second part of the question asks if having a plan is effective in protecting growers. There were several responses that addressed this first statement directly and they are recorded separately. The responses clearly indicate that this type of response of waiting for a pest to get to the farm is a dangerous strategy. Good businesses are prepared, and this usually results in a better outcome when the situation arises. This mixed response correlates with survey question Biosecurity 1 2 & 3.

   Does a good farm biosecurity plan and implementation protect growers?

   Review of Responses
   Most participants addressed this part of the question. There was a range of responses with the established regions being more supportive of biosecurity plans helping keep farms safe from incursions. In areas with small growers or challenging factors such as crowded growing areas in Carnarvon, there was a degree of hesitation in endorsing biosecurity plans as an effective protective mechanism. Again, this highlights the effect of having access to support in the farm planning and decision-making processes.
   There is clear correlation with the mixed responses to survey question Biosecurity 1 2 & 3. The survey and discussion responses demonstrated knowledge of on-farm biosecurity and risk pathways (KASA) but was very varied in the intention to change current practices. (Practice Change). Enterprises with effective farm biosecurity systems were probably more open to change and improvements to build resilience than those that had lots of excuses as to why they can't have one.

   Recommendations arising

   - Farm biosecurity planning needs to be appropriate to small farmers to encourage uptake.
   - Small growers and NESB farmers need support to develop farm biosecurity systems that are achievable and add value to their enterprises.
   - Farm biosecurity planning needs to integrate with IPM systems to build resilience.

RESPONSES to the Group discussion questions for Workshops or farm visits.

Where to from here?

1. **Leaf Miner grower guides being updated and republished for vegetables, melons, onions, and potatoes (separate levies)**
   Are they useful and informative or too complicated, hard to find and a waste of time.

   Review of responses.

   Darwin Responses

- Useful as there are always new pests emerging, new technologies being developed. Updated info is handy for better mgmt.
- Needs to be up to date & managed (at least annually).
- Needs to be accessible.
- Alternative is being ignorant. Not so much whether they are useful, but format, accessibility, currency etc. is essential.
- Useful resource but need to be better promoted/distributed.
- More concise and user friendly.
- 5–6-page A4 pamphlet with pictures/diagrams is good.
- As researchers, we found these guides are very useful for us to give growers advice related to biology, management, and control.

Innisfail – Hmong Group Response

- Yes please, lots of pictures and videos.

Ayr Responses

- Why? Online access. Most up to date info always online.
- Online.
- Reason to go to it.
- Diagnostic.
- Translate.

Bowen Responses

- Not a waste of time, valuable source of information.
- Useful, good information on pest scouting and management.
- Would like to know more about severity on crop by crop.
- Use current update online.

Geraldton – Combined response from 4 Grower visits

- Useful – all 4 wanted copies translated into Vietnamese.

Carnarvon – Workshop Floor Responses

- Need it in Vietnamese, more videos.

Carnarvon – Carnarvon Growers Association (CGA) President – grows vegies and fruit.

- We put out material through our CGA depot.
- Will distribute new guides, need them in Vietnamese.

Carnarvon – Carnarvon Vietnamese Community Association President – ex vegie grower

- Growers need useful information.
- Please translate to Vietnamese when finished.

Carnarvon – Organic Grower (left at start of the presentation)

- Yes, look at AUSVEG website for information.
- Will check out updates.

Carnarvon – Grower visit – Capsicum and Chilli – Protected cropping – Shade Cloth
- Need to be translated to Vietnamese, good photos.

Broome – DPIRD Response
- Need to know they are available.

Broome – TAFE Horticulture Response
- Very useful in training and keeping up to date.
- Need to get on distribution list for AUSVEG.

Broome – Asparagus Grower Response
- Get our info from the company agronomist.

Broome – Grape Grower Response
- Need to check with our agronomist.

Broome – Small Vegie Grower Response
- Yes, but not much time to keep checking.
- Rely on departmental officers.

Kununurra Responses
- Very useful
- Grower guides are very good.
- They seem to have enough detail without going overboard.
- Sometimes too many words and not enough pictures.
- Yes important.
- More so to understand beneficials and harshness of chemistry.
- They are very useful.

Bundaberg Workshop Responses
- Not waste of time, very useful, a bit complicated
- As long as info is available on the website, is informative and grower friendly, it is worth continuing.
- Guides are an essential tool on farm.
- Younger growers are more switched on and accept new ideas and technology.
- Yes, and useful. Thank you.

Bundaberg – Combined response from 3 Grower visits
- Grower 1 – yes please and for our Agro's
- Grower 2 – Need more info on management that fits with IPM.
- Grower 3 - Okay.

2. **Rapid accurate diagnostic options now available in local departments and potentially to industry.**
   Do the techniques shown at the workshops offer better options for management?

Darwin Responses

- Of course, very useful! These diagnostic options will be very helpful to help with early detection of the species, particularly when they are too small to identify. This helps with making control decisions!
- Provide a good option for identification, which can then guide management.
- It would help in early detection of pest.
- Possibly for Agro's, however I am not one.

Innisfail – Hmong Group Response

- Will go to QDAF, now we have a contact.
- Find out if Fall Armyworm attacks Taro – sent on to Masimo.

Ayr Responses

- Basic diagnostic is all that's required – management is still the same (Chemical).
- Yes.

Bowen Responses

- Yes – maybe a little expensive.
- Great for technicians in the field but needed with experience.
- For Leaf miners useful.
- Fall Armyworm/ Heliothis not so much.
- Not available in this region (Bowen/Ayr).
- Rapid test is more useful for new locations when SLM/ASLM has not been detected yet.
- Useful as diagnostic/confirmation tool.

Geraldton – Combined response from 4 Grower visits

- Refer samples to DPIRD.

Carnarvon – Workshop Floor Responses

- Ask Department when new damage is found.

Carnarvon – Carnarvon Growers Association (CGA) President – grows vegies and fruit

- The department (DPIRD)should do this.
- Good to have early diagnosis but need to know what to do then.

Carnarvon – Carnarvon Vietnamese Community Association President – ex vegie grower

- Need CGA to find out how.
- We get support from DPIRD research station.
- Good if they will check for us.

Carnarvon – Organic Grower (left at start of the presentation)

- Diagnosis is important but monitor regularly so will see new damage.
- Will ask CGA to check when new/unknown damage is seen.

Broome – DPIRD Response

- Not sure how we could support diagnosis.

Broome – TAFE Horticulture Response

- Yes. Students need to know new technology for ID and treatments.

Broome – Asparagus Grower Response

- Doesn't make any difference.

Broome – Grape Grower Response

- Can we get the department to do this in Broome?

Broome – Small Vegie Grower Response

- Get the department to check.
- Has had NAQS surveys in the past, good to learn from.

Kununurra Responses

- Yes. Need to know what we have.
- May be early diagnostic with tools such as LAMP for Fall Army Worm.
- It can be useful for other pests sp. which are hard to discriminate.
- Yes. Relevant especially to research i.e., proper id.
- Useful for biosecurity but not necessarily for management decisions.
- Could be good for pathogens more than insects.
- Crop and pest specific, sometimes it does not matter what the exact species it is.

Bundaberg Workshop Responses

- Useful, but it is a cost.
- Might be useful if regional data can be shared in a timely manner regarding species/ time of year.
- Rapid and accurate diagnostic options are ideal, currently specialist labs are expensive and cost prohibitive for growers.
- No.

Bundaberg – Combined response from 3 Grower visits

- Grower 1 – how do our small growers get access to diagnostics?
- Grower 3 – Maybe.

3. **Does the requirement to protect the parasitoids for leaf miners and for FAW fit with the other pest management requirements on the local vegetable and other affected farms?**
   Can we make it work?

Darwin Responses

- IPM works for most of the pests. Will require more targeted practices.
- Yes, Biocontrol can be cheap!
- Essential for further research.
- Need to find a way to make it work as chemicals will inevitably fail.
- Can make it work with correct education around product choice and timing.
- Yes, it does. IPM should be designed to work for the whole crop so it can be suitable to manage all different insect/pest on the crop.

Innisfail – Hmong Group Response

- Look at suite of beneficials in the area.
- Work with QDAF to identify especially in in Tropical Fruit i.e., Fruit Spotting Bug etc. parasitoids.

Ayr Responses

- NO. Minimal tolerance for crop damage.
- Systems.
- Local Population.
- Generated by retailers.
- Migration too fast.
- Question market adjustment.
- Just another grub, no major problem.
- More strategic Group 28 application, poor application is the problem.

Bowen Responses

- Yes – there does need to be much more education in this area.
- Need more work on beneficial parasitoids for Fall Armyworm.
- Need to work out how to mass rear the beneficials for releasing.
- Can work but hard under high pressure.
- Fall Armyworm in sweet corn must be treated to damage (0% tolerance for Fall Armyworm damage/infest).
- Same for Leafy crops (0% tolerance)
- Trap crops, buffers, vegetative buffers to support parasitoids/predators?
- For implementing IPM it is important to protect the beneficials
- Less reliance on broad spectrum OP/Carbamate is critical.
- Mandatory sprays for market access is an impediment.

Geraldton – Combined response from 4 Grower visits

- Grower 1 – Yes, already an IPM grower.
- Grower 2 & 3 – No, regular spraying.
- Grower 4 – Yes, already IPM with beneficials.
- Growers 2 & 3 use medium impact chemicals, see permit table.

Carnarvon – Workshop Floor Responses

- Most growers spray regularly for best quality.
- Only a few use IPM strategies, mostly for caterpillars.
- Don't monitor or check for beneficials (don't know which are beneficials) so spray all insects.

Carnarvon – Carnarvon Growers Association (CGA) President – grows vegies and fruit

- Most growers just spray regularly.

- Markets paranoid if any insects in produce, so little IPM.
- Fruit fly eradication process caused problems with trusting government advice.

Carnarvon – Carnarvon Vietnamese Community Association President – ex vegie grower

- Most growers spray all the time.
- Not much IPM under shade cloth.

Carnarvon – Organic Grower (left at start of the presentation)

- Yes, I use IPM for my farm with mostly organic sprays.
- River corridor the only good bio-refuge around.
- Currently use soft chemicals where possible not IPM.
- Not sure if parasitoids can get into net. (found Spiny Shouldered Shield Bug on Capsicums).

Broome – DPIRD Response

- We are trying to get commercial growers to use more integrated pest management. Then the IPM will work.

Broome – TAFE Horticulture Response

- Yes, we try to use IPM and beneficials.
- Native Bush Tucker Garden is a good bio-refuge for things near vegie garden.

Broome – Asparagus Grower Response

- Trying to change to more sustainable pest control.

Broome – Grape Grower Response

- Trying to change to more IPM but will need to get control of Cluster Caterpillar.

Broome – Small Vegie Grower Response

- Yes.
- Found predator shield bugs in Thai Basil.
- Can't spray broad spectrum.
- Need an answer for grasshoppers.
- Cluster Caterpillar IPM is slow.

Kununurra Responses

- Yes, we already do this.
- Yes, there is already a lot of IPM used in different crops and systems.
- More education, trials and research are needed.
- Yes. Experience has suggested limited chemical efficacy in high numbers.
- Need other options (cultural etc.).
- Yes, it can work.
- We often find that having a balanced environment means some damage. But it's affordable and sustainable.

Bundaberg Workshop Responses

- Not workable on a large scale in the Bundaberg region.
- On individual grower levels it might be possible.
- No brainer – yes.

Bundaberg – Combined response from 3 Grower visits

- Grower 1 – Not a problem as we don't use IPM.
- Grower 2 – Need robust IPM systems to be sustainable.
- Grower 3 – Didn't work in capsicums. Need to find out how Fall Armyworm gets into fruit when no egg masses on leaf.

4. **Does the table of current permits for leaf miners offer alternatives for management? Will it be useful when deciding a response to these pests?**

Darwin Responses

- Value in focusing on the chem side, however table is a little confusing.
- More info is better than the alternative.
- Yes. Handy guide with options to save time.
- Education around chemical choice and application still important in addition.
- Yes, it does. Yes, this table will be very helpful for the growers to make the decision which group should be used to minimise the effect on beneficials.

Innisfail – Hmong Group Response

- Yes, gives us an idea of soft to hard (broad spectrum) chemicals as well.
- Which chemicals to start with.

Ayr Responses

- Yes, sheets like this are very useful for advisors.
- Yes, beneficial.
- Surveillance.
- Human error.

Bowen Responses

- Yes
- Don't focus on Leaf Miners, Fall Armyworm still needs attention.
- Try to get more chemicals for Fall Armyworm and get permits.
- Yes, helpful for permits.
- No, Group 28 products have proven to be ineffective, group 5 (Spinosat & Success are the SAME product)
- All others are only suppression.
- Make decision depending on the pest pressure and crop age.
- Consider predators to make a decision to look after.

Geraldton – Combined response from 4 Grower visits

- Yes, good to have current permits, includes many chemicals currently being used by those growers.

Carnarvon – Workshop Floor Responses

- Table very useful when/if pest gets there. Need legal sprays.

Carnarvon – Carnarvon Growers Association (CGA) President – grows vegies and fruit

- Yes, good to have a range of chemicals permitted for legal spraying if it gets here.
- We can then sell growers the correct sprays.

Carnarvon – Carnarvon Vietnamese Community Association President – ex vegie grower

- Good that growers can use chemicals legally.

Carnarvon – Organic Grower (left at start of the presentation)

- Couple there I can use.
- Yes, use most of these chemicals anyway.

Broome – DPIRD Response

- Yes, if we need it.

Broome – TAFE Horticulture Response

- Yes. Show students the correct use of insecticides.

Broome – Asparagus Grower Response
- Need to go to our agronomist.

Broome – Grape Grower Response
- Could be helpful if we get it.

Broome – Small Vegie Grower Response
- Don’t need it yet. Could be helpful.

Kununurra Responses
- Yes, it is helpful, especially when it is to avoid damaging the natural control mechanism.
- Yes. Need to understand residues and legalities.
- Yes. It’s really good to see the table with the impact on beneficials.
- Yes, as some crops require zero damage.

Bundaberg Workshop Responses
- Yes?
- Yes, Co-formulations are more effective.
- Self-experimentation with low rates of ‘registered’ co-forms may be an option.
- Yes.

Bundaberg – Combined response from 3 Grower visits
- Grower 1 - Need to comply with market requirements, so good.
- Grower 2 – Permits are good as need to have legal options.
- Grower 3 – Not many chemicals work on caterpillars inside capsicums.

5. **“We are not going to worry about it until it gets here. There is too much else to do.” What is your response to this very understandable statement?**

Darwin Responses

- High risk response.
- ‘The only thing certain in life is change’.
- Better to be prepared when it arrives.
- We do not agree! We should have preparedness so that we can be able to contain any future incursion.

Bowen Responses

- Farmers need to know what is coming to prepare.
- No preparation will cause poor response.

Bundaberg Workshop Responses

- Continue surveillance.
- ‘Business’ farmers do not prescribe to that theory, they are open to new ideas, technology and IPM.
- Keep monitoring but understand this statement.

Does a good farm biosecurity plan and implementation protect growers?

Darwin Responses
- Yes.
- Yes, it really does.

Innisfail – Hmong Group Response
- Farm Biosecurity, need to start thinking about protecting our farms.

Ayr Responses
- Farm biosecurity can only protect growers from a small number of pests. Flying pests will come in regardless. Soil biosecurity issues should be minimal unless doing dangerous things e.g., share equipment.
- Find out about Leaf Miners before they get there, focused on biosecurity.

Bowen Responses
- Good farm biosecurity is essential in preventing pest introduction on farm.
- It's really good to build good response system on farm like parasitoids and other beneficials.
- Biosecurity is critical in preventing the pest.
- Community/ Local Government wash-down facility
- We're trying, we need more non-chemical and chemical solutions.
- Better (faster on farm) diagnostics.
- Fast parasitoid introduction/support.
- PCR single use test for Leaf miner like Fall Armyworm.
- Take decision for long term stable solution.
- On farm biosecurity /farm hygiene should give protection from incursion/spreads.

Geraldton – Combined response from 4 Grower visits
- 2 growers have biosecurity in place.
- 2 growers will watch for it.

Carnarvon – Workshop Floor Responses
- Most growers do not have a biosecurity plan. Their focus is on managing pests for best quality production.

Carnarvon – Carnarvon Growers Association (CGA) President – grows vegies and fruit
- Biosecurity is a big problem.
- Farms all close together and bugs can move quickly to the next farm.

Carnarvon – Carnarvon Vietnamese Community Association President – ex vegie grower
- The area needs more effort on farm hygiene.
- Too many old crops left, and pests go from one farm to another.

Carnarvon – Organic Grower (left at start of the presentation)
- Always concerned about biosecurity.
- Big problem in Carnarvon with growers so close together and often leave crop residue.
- Try to control all insects, only input is seed.
- But neighbours are close.

Broome – DPIRD Response
- We try to discuss Biosecurity, but often the growers don't see the need as they are very isolated.

Broome – TAFE Horticulture Response

- Work on simple biosecurity plans for small growers if something gets here.
- Broome vegie growers are focused on green manure and in increasing soil carbon.

Broome – Asparagus Grower Response

- We are well isolated.
- Our problems come from the native bush.

Broome – Grape Grower Response

- We control who comes and goes on farm.

Broome – Small Vegie Grower Response

- Basic Biosecurity by isolation.

Kununurra Responses

- Yes, good farm biosecurity shows a professional approach to farming.
- Weeds the hobbyist from the true farmers.
- Biosecurity is very important.
- Prevention is better than cure.
- Be prepared.
- Depends on pest. Wasn't much we could do with Fall Army Worm.
- Yes, biosecurity is at the national and the farm level.
- It is of utmost importance to keep invasive pests out.
- A culture of being prepared and having options to manage problems is important.
- A plan is useful if it's used during a high-pressure situation. It stops panicked bad decisions.

Bundaberg Workshop Responses

- Yes, important to know what is about.

Bundaberg – Combined response from 3 Grower visits

- Grower 1 – Has not been much of an issue. Smaller growers are too busy.
- Grower 2 – We maintain very strict biosecurity on our farms.
- Grower 3 – Yes, need good biosecurity to protect farm.

Group discussion questions for Workshop or farm visits.

Where to from here?

1. Leaf Miner grower guides being updated and republished for vegetables, melons, onions, and potatoes (separate levies)
   Are they useful and informative or too complicated, hard to find and a waste of time.
   Comments

2. Rapid accurate diagnostic options now available in local departments and potentially to industry.
   Do the techniques shown at the workshops offer better options for management?
   Comments

3. Does the requirement to protect the parasitoids for leaf miners and for FAW fit with the other pest management requirements on the local vegetable and other affected farms?
   Can we make it work?
   Comments

4. Does the table of current permits for leaf miners offer alternatives for management?
   Will it be useful when deciding a response to these pests?
   Comments

5. "We are not going to worry about it until it gets here. There is too much else to do."
   What is your response to this very understandable statement?
   Does a good farm biosecurity plan and implementation protect growers?
   Comments

Appendix 4.4

Monitoring and Evaluation Report

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

Greg Owens (Consultant)

Image: Kununurra workshop October 2003

Monitoring and Evaluation Report

MT20005

Table X. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
< Refer to the M&E Plan >	< Identify aspects of project performance that address the Key Evaluation Questions >	< List opportunities for improvement and future development >
To what extent has the project achieved its expected outcomes? **End of project outcomes** (from project logic) 1. Increase in knowledge of SLM and ASLM and how Integrated Pest Management can improve the management of SLM with minimum use of insecticides.	There is clear evidence from the Cesar interview survey (appendix 2.3) and the Nth Australian workshop series group discussion responses to Q2 &3 (appendix 4.3) of an increase in knowledge on how to use IPM to improve the management of the exotic leaf miner flies. This was especially evident in regions that had received multiple engagements by the project. The discussion Q4, on the use of currently permitted chemicals in terms of impact on beneficial insects and hence management outcomes, demonstrated an increase in knowledge of possible consequential negative outcomes from the use of different chemical groups.	• Continuous engagement Locations with multiple engagements by MT20005 and MT16004 clearly showed that the level of understanding and implementation was dependant on repeat exposure to the key IPM messages of these projects. This engagement and support needs to continue so all regions develop robust and responsive IPM farming systems for current and future chemical resistant pest incursions by building on current knowledge and experience, regardless of the starting level of understanding. Most VegNET regional plans include IPM which provides a strong existing vehicle for this continued engagement.
2. A better understanding of the role of beneficial insects in IPM practices.	The improvement in understanding of the role of beneficials was evident from all participants. Agronomists demonstrated a sophisticated understanding of beneficial insects in farming systems and openly shared their thoughts on integrating the IPM needs for SLM and ASLM into current growing systems. (appendix 2.3 & 4.2) Recent experience from the participants with FAW IPM strategies reinforced this understanding of beneficials in farming systems. Growers understanding of these principles varied from nil to well experienced in implementing IPM. All growers engaged in Nth Australia reported a better understanding of the role of beneficial insects in IPM systems.	• Updating monitoring and further developing diagnostics tools The monitoring and diagnostic tools developed in these projects need to be continuously updated. LAMP primers for all exotic leaf miners are required. • Review of permitted chemicals As more efficacy and chemical resistance data becomes available the current permits will need to be reviewed and those appropriate to the ongoing IPM strategies renewed.
Intermediate Outcomes 1. Increased awareness what effect insecticides have on SLM and ASLM and the parasitoids that use them as hosts.	Nth Australia Discussion Q4 demonstrated the increased awareness of the effect of insecticides on the leaf miner parasitoids. The Cesar interview survey in NSW highlighted the need for clear advice in the use of chemicals to manage the pests which shows that agronomists are aware of the ramifications of insecticide use on these pests.	• Continued chemical resistance and efficacy research The full suite of resistance to insecticides still needs to be determined especially against newer, softer, and more targeted chemical options. Efficacy data on more insecticide groups needs to be investigated to provide more

		options to commercial produce with minimal damage specifications.
2. Being able to identify the active SLM and ASLM populations by looking at leaf mines, using monitoring tools and rearing techniques.	The Qld, NT and WA workshops used local examples of leaf mines where possible, with preserved pest and parasitoid samples and used field and high-powered video microscopes to instruct growers and agronomists. This approach was very effective with the Vietnamese growers in Carnarvon.	• Survey different vegetable producing regions to determine what parasitoids are present attacking local native leafminer populations.
3. Improved management by identifying gaps in available chemistry, integration with current management practices and resistance issues	Nth Australia Discussion Q4 highlighted the range of expertise in implementing IPM strategies for leaf miners and other exotic pests such as FAW. There is anecdotal evidence from Survey Q3,4&5 that attendees at workshops considered that IPM approaches are working but they need to be underpinned by regionally specific information. The efficacy and application of newer chemicals was highlighted in Discussion Q4. Ready access to, and use of improved diagnostics Discussion Q2 was seen as desirable in most regions.	• Continued research into more chemical options that will play a positive role into IPM management strategies.
How relevant was the project to the needs of intended beneficiaries?	The project continues to gain in relevance with the continued spread of SLM through the southeast of Australia and of ASLM in North. Without guidance, growers when first encountering these pests, can easily increase the severity of the incursion by inappropriate management. Nth Australia Survey Q13 and Discussion Q1 almost unanimous support from growers and industry support for committing their levy money to ongoing related projects and providing updated information products. There were strong requests for translation of materials to increase the relevance to the NESB growing community.	• Monitor continued spread Due to the continued spread of SLM and ASLM into other growing regions, it is necessary to increase monitoring of pest and parasitoids and industry engagement to ensure newly affected areas have access to regionally relevant information and management options. • Continuously updated IMP and extension materials Growers and agronomists were fully supportive of continued improvement in information available for leaf miner management.
How well have intended beneficiaries been engaged in the project?	The communication and engagement strategy produced in MS102 provided for a comprehensive engagement program across all mainland Australia vegetable and melon growing regions and using a range of engagement tools. The project utilised Webinars during the end of Covid and then used 2x conferences, 2x field days, 3x field walks, 44x farm visits, 3x market visits, 7x agronomist meetings, and 16x formal workshops. The project recorded 351 direct attendees over these activities, not including the conferences and field days. 227 growers and agronomists attended the formal workshops. Regions with a history of engagements with the project and the previous project MT16004, demonstrated a greater knowledge of appropriate management and had adopted or displayed willingness to adopt improved management strategies. They recorded extensive	• Continued Engagement Mature growing areas need the opportunity to discuss and question best practice management that evolves from continued research. NESB growers need support, possibly through VegNET Regional Officers to develop more sophisticated Biosecurity and IPM focus. Survey Q3,4 and B1,2, &3

	knowledge of and use of previous extension materials Survey Q 6 – 9. The enthusiastic response to farm visits and workshops from NESB growers in Geraldton, Carnarvon and Innisfail indicated they want more engagement in this space, Survey Q15. When presented with the extension material from MT16004 and MT20005 they were eager to incorporate the new knowledge but also wanted resources in language, if possible. Survey Q6-10 and Discussion Q1	
To what extent were engagement processes appropriate to the target audience/s of the project?	The early part of the project was still impacted by Covid restrictions, so the project produced webinars. This attracted the key growers and agronomists impacted in Qld and NSW. The interviews conducted by Cesar following these virtual engagements showed a high level of knowledge uptake and provided the project team with relevant questions from industry to be addressed by the project. Attendance at engagement activities in Nth Australia was recorded and clearly demonstrated that appropriate activities were selected and planned for each activity. For example, 95 growers and industry support personnel attended the Carnarvon workshop held in a leading growers shed and supported by the Carnarvon Vietnamese Association and Carnarvon Growers Association. Farm visits were more suitable in some locations and allowed in-depth engagement with individual growers. Nth Australia Q14 responses indicted almost all attendees at every event would attend subsequent activities.	• Continued Engagement Select and conduct appropriate engagement and IPM learning activities for leaf miners and other highly resistant insect incursions.
What efforts did the project make to improve efficiency?	There was a major variation in Oct 2021 following detection of ASLM in WA and NT to increase leafminer surveillance, parasitoid monitoring, and an expanded Nth Australia engagement program to include growers from Carnarvon WA to Bowen QLD. This allowed the project to build on the knowledge and techniques already being developed for SLM and used existing project networks and expertise.	• Continued engagement with NESB growers. Translation of documents into appropriate language and follow up engagement is necessary to build on preliminary gains in knowledge and attitude to an IPM approach.

Table 4: Additional evaluation data requirements

KEQ	Data collection requirement	Source and method
Did the implementation of IPM tools provide adequate control of SLM	*Survey of grower practice change as more information becomes available as a result of project outcomes and outputs.*	*Workshop presentations and evaluation questionnaires on material provided.*

and ASLM in terms of productivity and profitability.	Growers and agronomists in Kununurra and Darwin Rural, impacted by ASLM, have demonstrated the best outcome for control of ASLM comes from an IPM approach. A corresponding reduction in FAW pressure has also been noted from IPM strategies in these areas. The increased awareness of the negative outcome of a chemical only approach, in regions affected by SLM, has been documented in SE Australia. Products that require blemish free leaves face challenges, but the table of permitted chemicals offers some alternatives within an IPM system. Survey Q3 & 4 show that most agronomists are recommending IPM or softer chemicals as standard and have given this advice to growers for leaf miners and FAW. Responses to discussion Q 3&4 reinforce this with inciteful comments and discussion on IPM and chemical use.	Survey Q 2-5, Discussion Q 3,4
Did the use of the rapid in-field molecular diagnostic assay assist growers and agronomists in the identification of SLM and ASLM?	*Suspected SLM samples continue to be presented for identification.* Records of host crops increasing with preferred crops identified and demonstrated in a demonstration plot at the Smart Farm Field Day at DAF Gatton in Nov 2023 The response to discussion Q2 on the usefulness of rapid diagnostics demonstrated at the workshops was varied. There was a common theme that it needed to be accessible and affordable for growers and agronomists. There were also comments that it was much more applicable to Biosecurity surveillance activities, as the species didn't change the in-field management.	*Growers and/or agronomists rearing specimens for identification.* Discussion Q2
Did the project provide useful biological, cultural and chemical information relevant to the control of SLM and ASLM across enough commodities?	*Relevant material being sourced on AUSVEG website and other websites.* The survey Q5-9 asked specifically if attendees to the extension activities had seen, read, used, or would use and recommend the information provided by MT16004 and then discussion Q1 if an update for the grower guides was worth the effort and expense. While many of the agronomists and industry support group had at least seen the information products many of the growers surveyed had not. Once exposed to these products though the response was overwhelmingly positive on them being useful and would be used. The request	*Workshops and grower meetings.* *Discussions with growers and agronomists.* Survey Q5-12 Discussion Q 3 & 4 Nth Australia Extension report

	for translations into Vietnamese for NESB growers was across all northern regions. The biological information provided by using live local samples where available and preserved specimens presented with field and high-powered microscopes and high-quality photographs were well received at these workshops. This was demonstrated by the enthusiastic engagement in these activities. The discussion Q3& 4 gave participants a chance to incorporate the information provided and to consider how this information would impact on their current or planned insect management systems. It asked specifically how the information on the required IPM approach provided would fit with their current cultural practices. The response showed that the participants understood what was presented and had started to think how it could be incorporated into their current practices. The table of permitted chemicals that was distributed during the Nth Australia workshops and at the Gatton Smart Farm field day was well received by growers as providing appropriate advice to the legal use of chemicals on leafminers. The chemical resistance information provided by NSW DPI further refined this information as to those chemicals identified as less than useful due to high levels of resistance in the exotic leafminers. The responses to discussion Q3&4 both demonstrated this information provided in the workshops was incorporated into their deliberations.	
Have regular project updates been provided through linkage with the industry communication project eg VEGNET?	*Regular project updates provided through industry communication project (VEGNET) and other grower newsletters (Vegetables Australia) and websites.* Get data from AUSVEG comms unit. For Veg and Potatoes. 2 articles for melon news were provided. https://www.melonsaustralia.org.au/news-and-resources/publications/magazine Management Strategy for Serpentine Leafminer, June 2023 Alternate Hosts for Serpentine Leafminer, Dec 2022	*Provision of published industry communication articles and relevant meeting and conference documents.* AUSVEG website Melon news
Were project outcomes	*Number of articles/presentations provided to*	*Provision of published industry*

<table>
<tr>
<td>provided in a readily accessible form to stakeholders?</td>
<td>stakeholders in an accessible format.

The responses to the Cesar interviews in SE Australia indicated the information provided was well understood and led to in-depth discussions on implementation of IPM strategies to manage SLM.

The information presented at the workshops across Nth Australia and in the Lockyer had a high degree of acceptance as demonstrated by the answers to survey Q5-9 on the information materials from MT16004 and the response to discussion Q1 which endorsed the preparation and distribution of an updated grower management guide.

When questioned growers and agronomists saw the preparation of these materials and the extension engagement program as good use of their levy money and were happy to have more levy money dedicated to these outputs.

Incorporating practical sessions in the workshops with local samples of the pests and damage or preserved specimens increased the engagement and understanding of the management of the exotic leaf miners.

These resources need to be translated to appropriate languages and actively distributed and supported through existing networks.</td>
<td>communication articles, grower guides and relevant meetings and workshop handouts.

Survey Q6-9, 11,15

Discussion Q1

Nth Australia Extension report</td>
</tr>
<tr>
<td>How effective was engagement with the vegetable, potato, onion and melon industries?</td>
<td>Feedback from local grower groups as to the assistance provided them to manage this pest.

The project was effective in engaging growers across Australia as shown in this attendance summary.

Condensed summary of extension activities
<table>
<tr><th>Event</th><th>Number of events</th><th>Attendance</th></tr>
<tr><td>Growers' expo</td><td>2</td><td>45+</td></tr>
<tr><td>Field days</td><td>2</td><td>600+</td></tr>
<tr><td>Field walks</td><td>3</td><td>25</td></tr>
<tr><td>Webinars</td><td>3</td><td>56</td></tr>
<tr><td>Farm visits</td><td>44</td><td>68</td></tr>
<tr><td>Grower meeting</td><td>1</td><td>12</td></tr>
<tr><td>Workshops</td><td>16</td><td>227</td></tr>
<tr><td>Agronomist meetings</td><td>7</td><td>25</td></tr>
<tr><td>Melon roadshow</td><td>2</td><td></td></tr>
<tr><td>Market visits</td><td>3</td><td>3</td></tr>
</table></td>
<td>Number of participants at meetings and workshops.

Attendance data from extension program

Survey Q15</td>
</tr>
</table>

	Conferences | 2 | This summary demonstrated the breadth of engagement types used to get the project messages to the affected industries. Survey Q15 recorded almost universal intention to attend more similar workshops or extension events held across Nth Australia.	
Was the information presented in a way that was useful to growers?	*Feedback from local grower groups* Growers given the information products from MT16004 clearly indicated that they would use these resources and share them with their neighbours in Survey Q 8&9. A concern is the number of growers that had not seen the material previously. There was a noticeable legacy effect in regions where workshops or other engagement activities concerning leaf miners were conducted previously. The response to Discussion Q1 on whether an updated grower guide would be worth the effort and expense was a very positive "yes" from the growers. The key learnings from the extension effort across Nth Australia were that there needs to be an active program to get these support materials to growers when they are available and this could be better coordinated through VegNET Regional Development Officers, who have the local networks.	*Growers and agronomist from locally e infested regions.* Survey Q 8&9 Discussion Q1 Key learnings Nth Australia Extension program
What has the project achieved to assist growers manage SLM and ASLM?	*Feedback from local grower groups* Growers and agronomists pointed to the chemical resistance and abundance of native parasitoids as the key information in the current and proposed management guides and provided by the workshops and other engagement activities. These two key points underpin the need and the observed success of using IPM strategies to manage these pests. Regions yet to be affected by SLM or ASLM are aware of the symptoms to look for and the likely timing in their climate from the Cesar modelling. They are also armed with the knowledge on which of the few chemical groups are still effective and have had a chance to consider how the required IPM management would fit into their current pest management. The project was able to relate real, current, positive IPM experiences from affected areas and growers. The importance of biosecurity as a way of	*Growers and agronomist from locally infested regions.* Survey Q 8,9, B1-3 Discussion questions 2-5 Key learnings Nth Australia Extension program

	farming was incorporated and responses to Biosecurity Survey Q 1-3 indicated that agronomists have incorporated biosecurity on-farm planning into their advice and that most growers have, or are moving toward on-farm biosecurity plans .	
To what extent has the project identified scientific or knowledge gaps that require future prioritisation and investment?	The project identified a number of gaps still existing in the scientific knowledge relating to the exotic leaf miners that have made it to Australia. These are included in Section 8 of the draft Industry Management Plan for Exotic Leaf miners. Gaps remaining include more chemical resistance data, how to encourage or commercially produce effective parasitoids, impacts of newer chemistry on pest and beneficials, incorporating spread and climate impact data from modelling into regional IPM strategies, managing leafminers in protected cropping, managing weed hosts for pest and beneficials and refining existing IPM practices based on regional data.	*Provided through final report.* Research Gaps in section 8 of the IMP

Project Logic for MT20005

Rational against the Strategic Investment Plan

Relevant SIP outcomes	**Improved farm productivity – Pests and Diseases - Protect the vegetable industry from both endemic and exotic pests and diseases that significantly damage the industry.** **Vegetable (SIP) 2017-2021** Continue with a prioritised R&D program to manage pest and disease challenges and threats with a focus on soil health and IPM Onions SIP 2017-2021 **Average yields have significantly improved, resulting in reduced cost of production - Integrate precision ag, integrated pest management (IPM) and soil health as core elements of the potato extension program** **Potato Grower SIP 2017-2021** Accelerating widespread access to and use of existing R&D findings and proven management practices that will help growers to reduce the costs associated with pests, weeds and diseases Melon SIP 2018-2021 **Improved industry protection from exotic, emerging and endemic pests and diseases** **Nursery SIP 2017-2021**

Expanded Project Logic with evidence sources.

Deliverables

End-of-project outcomes	Increase in knowledge of SLM and ASLM and how Integrated Pest Management can improve the management of SLM with minimum use of insecticides. A better understanding of the role of beneficial insects in IPM practices.

End of Project Outcomes

1. Increase in knowledge of how IPM management of SLM and ASLM with minimum use of pesticides.
   - Research (Technical- published information and recommendations)
   - Extension (KASA- Workshops, Survey Q3 discussion), CESAR survey
2. A better understanding of the role of beneficial insects in IPM practices
   - Research (Technical – published information)
   - Extension (KASA- Workshops, Q3 discussion), Cesar Survey

Intermediate outcomes	Increased awareness what effect insecticides have on SLM and the parasitoids that use them as hosts.	Being able to identify the active SLM populations by looking at leaf mines, using monitoring tools and rearing techniques.	Improved management by identifying gaps in available chemistry, integration with current management practices and resistance issues

Intermediate outcomes

1. Increased awareness what effect insecticides have on SLM and ASLM and the parasitoids that use them as hosts.
   - Research (Technical – published information)
   - Extension (KASA- workshops, Q3&4 discussion), Cesar Survey
2. Being able to identify the active SLM and ASLM populations by looking at leaf mines, using monitoring tools and rearing techniques.
   - Research (Technical – published information, Monitoring section IMP)
   - Extension (Workshops- practical)
3. Improved management by identifying gaps in available chemistry, integration with current management practices and resistance issues

Foundational

- Research (Technical – published information, Gap analysis IMP)
- Extension (KASA- knowledge & application Q4 discussion), Cesar survey

Outputs	Diagnostic test and surveillance protocol for serpentine leafminer including published methodology for rapid in-field molecular assay to identify SLM	Interactive online portal containing seasonal SLM pest forecasts and visual pest and beneficial population spread outputs to support monitoring and management decisions.	Industry management plan for serpentine leafminer produced as well as 3 industry grower guides for vegetables, potatoes and onions.
	VegNet network - content developed for regional newsletters through the VegNet network and Industry magazine articles	Digital dissemination and availability of communication products made available through HIA and AUSVEG, Melons, GIA, VegNET websites.	Sampling guidelines, including local host range etc for growers and agronomists.

Outputs

1. Diagnostic test and surveillance protocol for serpentine leaf miner including published methodology for rapid in-field molecular assay to identify SLM and ASLM.
   - Research (Technical – published information, diagnostic techniques)
   - Extension (KASA- workshops, Q2 discussion)

2. Interactive online portal containing seasonal SLM and ASLM pest forecasts and visual pest and beneficial population spread outputs to support monitoring and management decisions.
   - Research (Technical – published information)
   - Extension (Survey Q2, 7-11)

3. Industry management plan for serpentine leaf miner produced as well as 3 industry grower guides for vegetables, potatoes and onions. Split leafy vegetables and fruiting vegetables and melons.
   - Research (Technical – published information, IMP)
   - Extension (Updated Grower Guides)

4. VegNET network - content developed for regional newsletters through the VegNET network and Industry magazine articles
   - Research (Technical – published information)
   - Extension (Survey Q2)

5. Digital dissemination and availability of communication products made available through HIA and AUSVEG, Melons, GIA, VegNET websites.
   - Research (Technical – published information)
   - Extension (Survey Q6 -10), Cesar Survey

6. Sampling guidelines, including local host range etc for growers and agronomists.
   - Research (Technical – published information, monitoring section IMP)
   - Extension (Survey Q6 -10) Cesar Survey

Activities

Activities				
	Validation of in-field diagnostic for flies and empty leaf mines including RPA or LAMP	Field surveys, identification of weedy reservoirs for SLM (QLD and NSW)	Validate survey guidelines in paddocks affected by SLM (low density)	Develop industry management plan
	Communication & Engagement plan, workshops and new extension material (eg grower guides)	Increase resolution of/validate the spread model and establishment model, access to incursion survey data.	Crop protection gap analysis (with local chemical use patterns data collection)	Field surveys for parasitoid identification

1. Validation of in-field diagnostic for flies and empty leaf mines including RPA or LAMP
    - Research (Technical – published information)
2. Field surveys, identification of weedy reservoirs for SLM (QLD and NSW)
    - Research (Survey results- published information)
3. Validate survey guidelines in paddocks affected by SLM (low density)
    - Research (Technical – published information)
4. Develop industry management plan
    - Extension and communication (Draft IMP)
5. Communication & Engagement plan, workshops and new extension material (eg grower guides)
    - Extension and communication (Published MS102)
6. Increase resolution of/validate the spread model and establishment model, access to incursion survey data.
    - Research (Technical – published information)
7. Crop protection gap analysis (with local chemical use patterns data collection)
    - Research (Technical – published information, IMP Gaps section)
8. Field surveys for parasitoid identification
    - Research (Technical – parasitoid ID's published)

Additional Activities

7. A series of extension events including workshops, farm visits and other engagement activities across Northern Australia following the detection of ASLM.
    - Extension Summary of extension activities (Nth Australia Extension activities table with attendance)

Activities

Extension activities summary

Condensed summary of extension activities

Event	Number of events	Attendance
Growers' expo	2	45+
Field day	2	600+
Field walks	3	25
Webinars	3	56
Farm visits	44	68
Grower meeting	1	12
Workshops	16	227
Agronomist meetings	7	24
Melon roadshow	2	
Market visits	3	3
Conferences	2	

Regions Visited

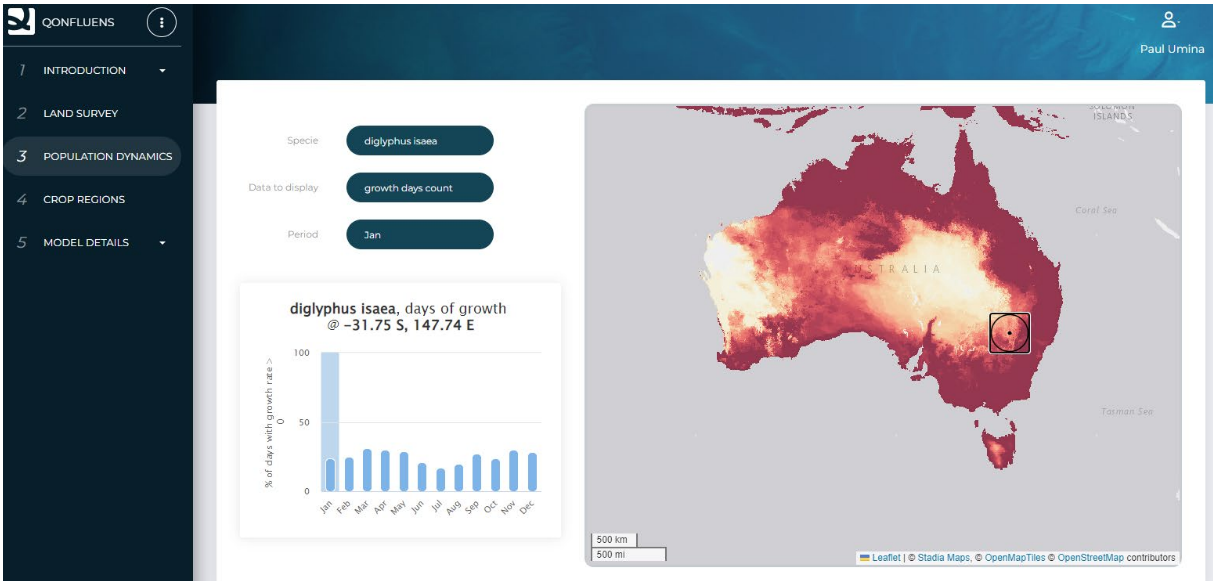
All growing regions in Australian mainland were visited for workshops, meetings, and farm visits. Multiple visits were made to the Nth Qld & SEQ.

Appendix 5.1

Summary of the modelling/app development within the Serpentine Leafminer Project

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by Virgile Baudrot – 2023



Summary of the modeling/app development within the Serpentine Leafminer Project

Virgile Baudrot – 2023

1. Modelling

1.1. Objectives of models

The main objective of the modeling tool is to better capture the link between the seasonal activity of Leafminer species and the seasonal activity of their Parasitoids so that growers and advisers can better understand and manage the population dynamics of the pest and its parasitoids. These processes vary across region and season so an approach that considers this spatial variation in conditions is necessary to provide insights that are timely and regionally relevant to affected plant industries. The diagram of the project described by Figure 1 consists of three steps:

1. Mapping collected data within a web-app (using for instance the R shiny web-app).
2. Prediction of leafminers and parasitoid populations using the model described in Figure 2 (using Julia libraries: GrowthMap.jl, DynamicGrids.jl and Dispersal.jl).
3. Connecting prediction with collected data on the web-app (using R shiny web-app).

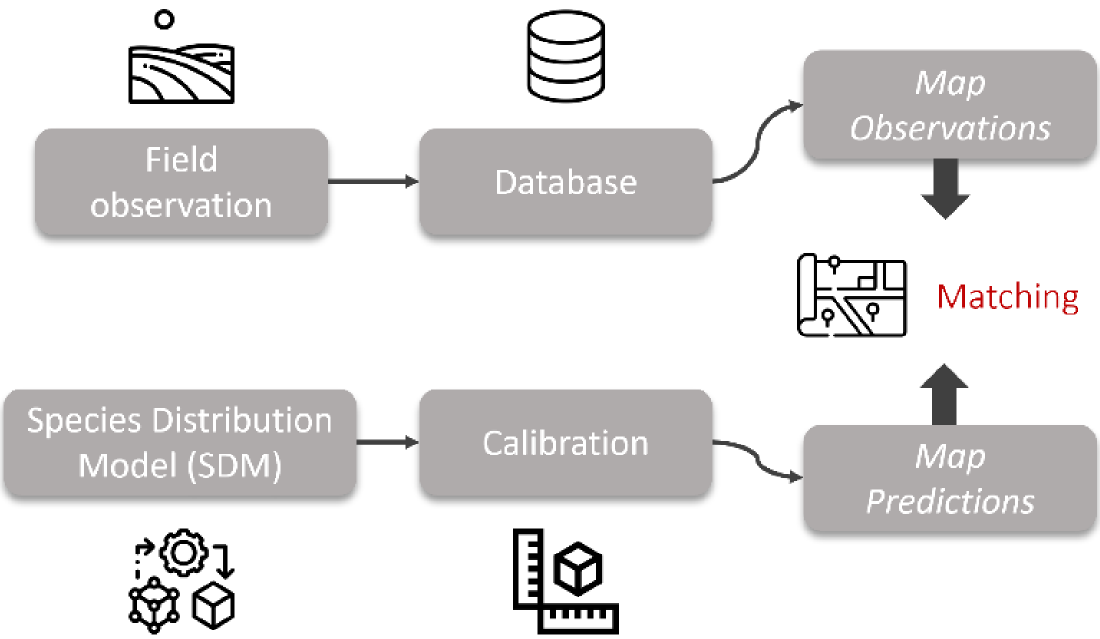


Figure 1 - Project diagram connecting Field Observations to Species Distribution Model (SDM).

1.2. Model diagram

The model diagram of Figure 2 provides an overview of the SDM (Species Distribution Model) given in the project diagram of Figure 1. Coloured rectangles are variables and grey boxes are rules. Variables and rules of the model are described hereafter.

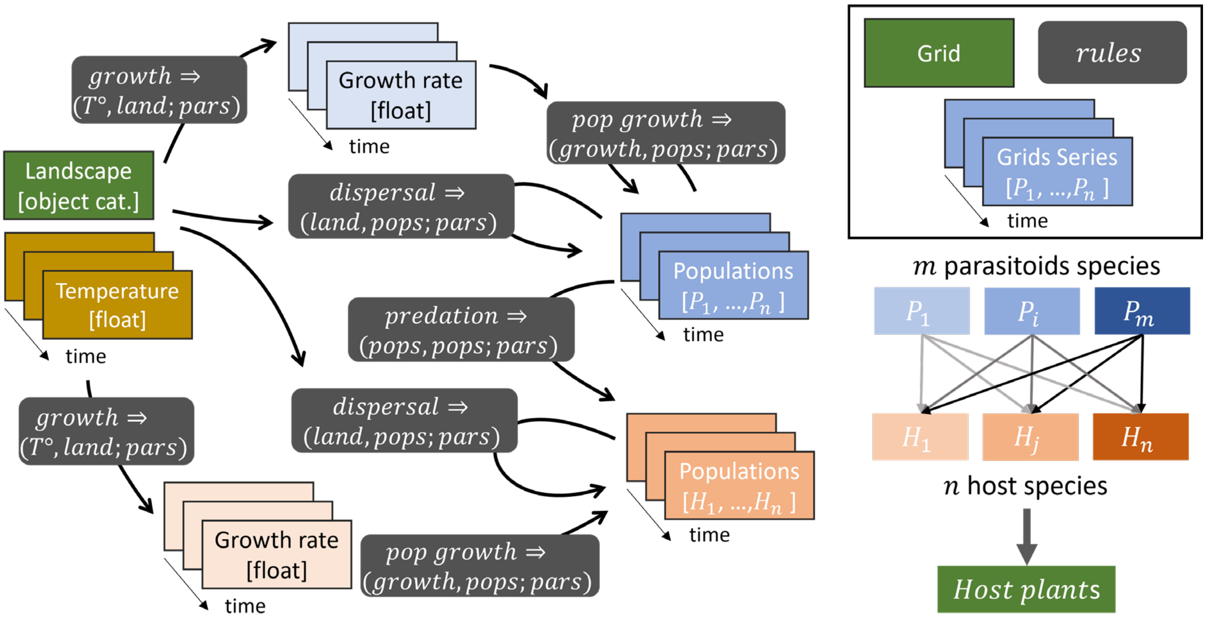


Figure 2 - Model diagram for the predictive host-parasite model. Coloured rectangles are variables computed over the grid (i.e., landscape composition, mean daily temperature, daily growth rate, and population densities) and dark-grey boxes are rules applied over variables to compute grids (i.e., growth function, dispersal, population growth, predation).

1.3. Species Variables

Table 1: Leafminer species

Species	Common Name
Liriomyza huidobrensis	Serpentine leafminer
Liriomyza sativae	Vegetable leafminer
Liriomyza trifolii	American serpentine leafminer

Table 2: Parasitoid species

Parasitoids	Natural enemy of	References
Diglyphus isaea	• *Chromatomyia horticola* • *Liriomyza bryoniae* • *Liriomyza trifolii* • *Pieris rapae* • *Plutella xylostella*	cabi.org
Dacnusa sibirica	• *Chromatomyia horticola* • *Chromatomyia syngenesiae* • *Liriomyza bryoniae* • *Liriomyza huidobrensis* • *Liriomyza trifolii*	cabi.org
Zagrammosoma latilineatum	• *Liriomyza huidobrensis*	cabi.org
Cirrospilus brevicorpus		
Hemiptarsenus varicornis	• *Liriomyza bryoniae* • *Liriomyza huidobrensis*	cabi.org

	• *Liriomyza sativae* • *Liriomyza trifolii* • *Ophiomyia phaseoli*	

1.4. Growth Rate and Stress Mortality

Temperature response parameter using Schoolfield et al. (1981) model given by the following equation:

$$\frac{p_{25} * \frac{x}{T_{ref}} * exp\left(\frac{H_A}{R} * \left(\frac{1}{T_{ref}} - \frac{1}{x}\right)\right)}{1 + exp\left(\frac{H_L}{R} * \left(\frac{1}{T_{0.5L}} - \frac{1}{x}\right)\right) + exp\left(\frac{H_H}{R} * \left(\frac{1}{T_{0.5H}} - \frac{1}{x}\right)\right)}$$

Table 3: Some parameterization (see also Maino et al., 2021)

Species	p_{25}	H_A	H_L	$T_{0.5L}$	H_H	$T_{0.5H}$	T_{ref}	Sources
Liriomyza sativae	0.20	5382	-82469	291	185564	307	25	Zhang 2000, Chien 2007, Haghani 2006
Liriomyza huidobrensis	0.043	6000	-10900	290	184000	298.5	25	Zhang 2000, Chien 2007, Haghani 2006
Liriomyza trifolii	0.148	7000	-104000	291.5	240000	305.75	25	Zhang 2000, Chien 2007, Haghani 2006
Diglyphus isaea	0.22	12000	-73500	286	282000	304	NA	Minkenberg 1989, Hondo 2006, Haghani 2007
Hemiptarsenus varicornis	0.22	24437	-225191	288	134917	303	NA	Hondo 2006, Chien 2004, Cheng 2017

Species	C_{Tmin}	m_{Tmin}	C_{Tmax}	m_{Tmax}	$C_{wilting}$	$m_{wilting}$
Liriomyza sativae	10 (5)	0.07 (0.1)	35	0.365	0.80	11.5 (10.5)
Liriomyza huidobrensis	-5	0.029	30	0.05	0.80	2.5
Liriomyza trifolii	5	0.07	35	0.2722	0.80	7.83
Diglyphus isaea	10	0.07	33	0.365	NA	NA
Hemiptarsenus varicornis	15	0.07	37	0.365	NA	NA

1.5. Predation

For parasitism we use a generalized predator-prey model following the system of equations:

$$\frac{dH_i}{dt} = \rho_i H_i - \sum_j \frac{a_{ij}H_i}{1+\sum_i a_{ij}h_{ij}H_i} P_j$$

$$\frac{dP_j}{dt} = -\gamma_j P + \delta_j \sum_j \frac{a_{ij}H_i}{1+\sum_i a_{ij}h_{ij}H_i} P_j$$

In this model, variable H_i is the host species i (i.e. a leafminer population) and the variable P_j is a parasitic wasp of species j.

Parameters of host populations are ρ_i for the intrinsic growth rate of the population (including growth rate and stress mortality defined earlier), and for the parasitic population, γ_j stands for the parasitoid mortality rate in the absence of food, δ_j is the conversion efficiency that convert food into new born. Finally the function $\sum_j \frac{a_{ij}H_i}{1+\sum_i a_{ij}h_{ij}H_i}$ is the multi-species functional response base which extends the Holling type-II functional response with: parameter a_{ij} is the attack rate of predator j and prey i, and the parameter h_{ij} is the handling time for predator j on prey i.

1.6. Dispersal processes

Many factors may affect dispersal processes which range from fully ecological to socio-ecological (e.g., human-assisted dispersal).

For a fully ecological dispersal, we assume a classical exponential dispersal kernel, with a shape parameter denoted λ ($\lambda = 0.25$ with radius of 1 cell). We add also an Allee-effect rule that requires a minimum of 20 founders to start a new population in cell (see Schouten et al., 2022).

For a socio-ecological dispersal, we assume a stochastic long-distance spatial process to mimic human-mediated transport events. For this rule, we have to define a range of cells on which the stochastic process will be applied (see Schouten et al., 2022).

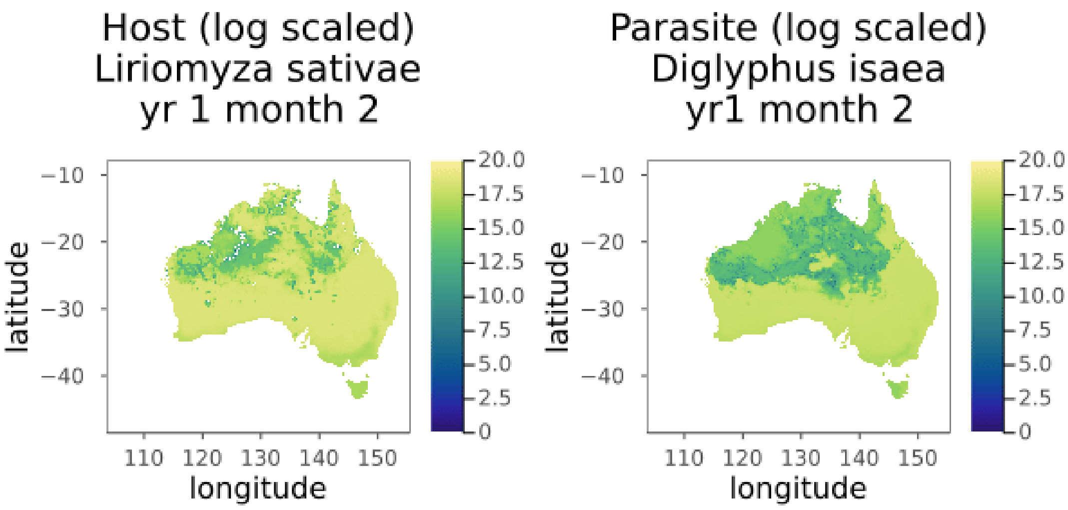


Figure 3 – Simulation of 10 years of dispersal of L. sativae (left) and D. isaea (right) when introduced everywhere in Australia.

1.7. Calendar

Table 4: Calendar of the project.

Tasks	2021 Q4	2022 Q1	2022 Q2	2022 Q3	2022 Q4	2023 Q1	2023 Q2
Model diagram (see Figs 1 & 2)	■						
Data aggregation seasonal tool	■	■					
Dev forecast seasonal tool		■	■	■			
Dispersal model			■	■	■		
Final deliverables	■		■		■		■

1.8. Manuscript

The model implementation and validation on data have been finalized through the article "Forecasting the potential distribution of invasive leafminer pests and their natural enemies"

Highlights of the article:

- Establishment potential of *L. huidobrensis, L. sativae* and *L. trifolii* in Australia, as well as two cosmopolitan parasitoid wasps known to provide control of the flies in both field and glasshouse settings, *Diglyphus isaea* (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae).
- Global distribution data spanning 42 countries were compiled and used to validate a process-based model of establishment potential based on intrinsic population growth rates.
- The modelling approach successfully captured the international distribution of the three *Liriomyza* species based on environmental variables and predicted the high suitability of non-occupied ranges in Australia.
- The largely unfilled climatic niche available to these pests demonstrates the early stages of their Australian invasions and highlights locations where vegetable production regions are at particular risk.
- In addition to Australia, our results highlight many regions globally where *L. sativae, L. trifolii* and *L. huidobrensis* have the potential to spread in the future.
- Within Australia, *D. isaea* and *H. varicornis* are predicted to have a large spatial and seasonal overlap with each *Liriomyza* species and thus are expected to influence the future spread of these pests and play an important role in local pest management programs.

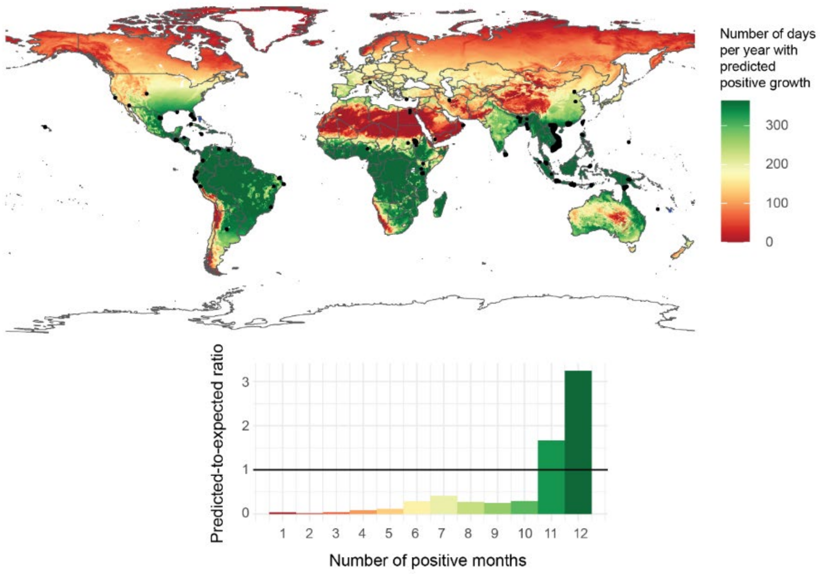
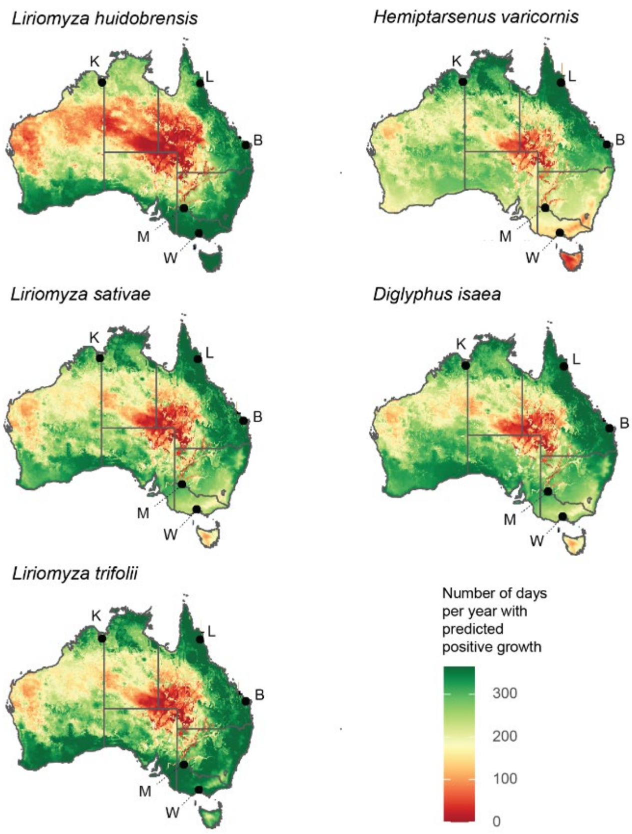


Figure 4: Global climatic suitability for L. sativae, depicted as the number of days during which positive growth rates are predicted to be achievable using climate data from 2017. Occurrence records of L. sativae (black dots) generally correspond to areas where the model predicts nearly 12 months of positive growth rate being achievable (see frequency graph inserted which shows the mean Boyce ratio by the number of months with positive growth in any given year). The Boyce Index as a measure of model goodness is 0.703, showing that the model predictions are consistent with the occurrence data.

Figure 5: Climatic suitability for L. sativae, L. huidobrensis, L. trifolii, D. isaea and H. varicornis across Australia, depicted as the number of days during which positive growth rates are predicted.



2. Web application

2.1. Objective

The objective of the application is to provide a tool to share results of the SLM project between members of the consortium and to help the production of visualization to communicate to farmers organizations.

2.2. Data Base

The objective is to find a standardized way to upload data in order to automatize the upload of new data within the application.

Access to the database:
https://docs.google.com/spreadsheets/d/1xv0rM_Y1KjtF70Jc8GOEdPhzhRD7Qd9MQC8E10oufsY/edit?usp=sharing

- 6 sheets where observations are recorded: (1) *wasp surveys SITES*, (2) *QLD leafminers sent for ID*, (3) *NSW parasitoids sent for ID*, (4) *QLD parasitoids sent for ID*, (5) *wasp surveys SPECIMENS, (6) Elia's existing data*
- 1 sheet used for the App: *SLM_DATABASE*

Table 5: The next table presents a ***suggestion for a standardized*** *way to add most important information. We can also add common names for species and hosts.*

Column name	Lat	Lon	Date	Species	Host
Examples of data	27.639760 S -27.42421 -27.549033 S	151.866368 E 152.473319	21 May 21 17/09/2021 05/15/2021	Liriomyza huidobrensis L. huidobrensis Opius spp (wasp)	Celery Wireweed (*Polygonum erectum*)
Validated	-27.42421	152.473319	17/09/2021	*Liriomyza huidobrensis* *Liriomyza* spp	*Apium graveolens* *Polygonum erectum*
Format	WGS84 EPSG 4326 Google Maps	WGS84 EPSG 4326	DD/MM/YYYY	*Genus species* *Genus* spp	*Genus species* *Genus* spp

2.3. Two apps: technological dilemma

We started by using the R shiny app technology to create the app. Then, we provided another version based on more common technologies (Flask, Docker, VueJs) in order to be able to integrate more complex features within the tool (e.g. user login to add their own data, running simulation from the app, …).

- This R shiny app can be find here: https://ec2-52-65-31-166.ap-southeast-2.compute.amazonaws.com/LeafMinerShinyObs/
- The Flask-VueJs app is here (login require): https://apps.qonfluens.com/platform/login

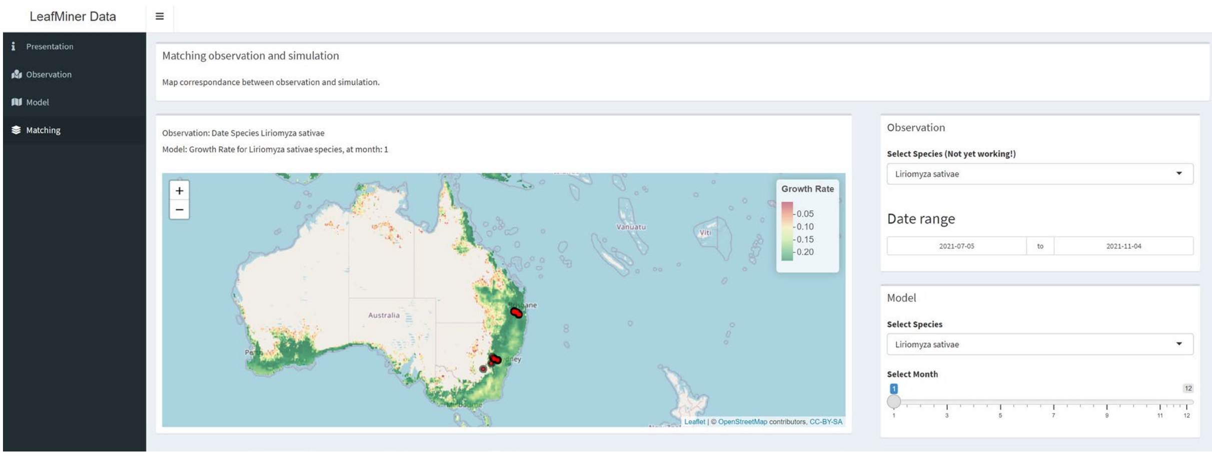


Figure 6: Screenshot of the R-shiny app. Matching item with the overlapping of observation data points (red points) with simulation map of potential growth rate (green to red colors).

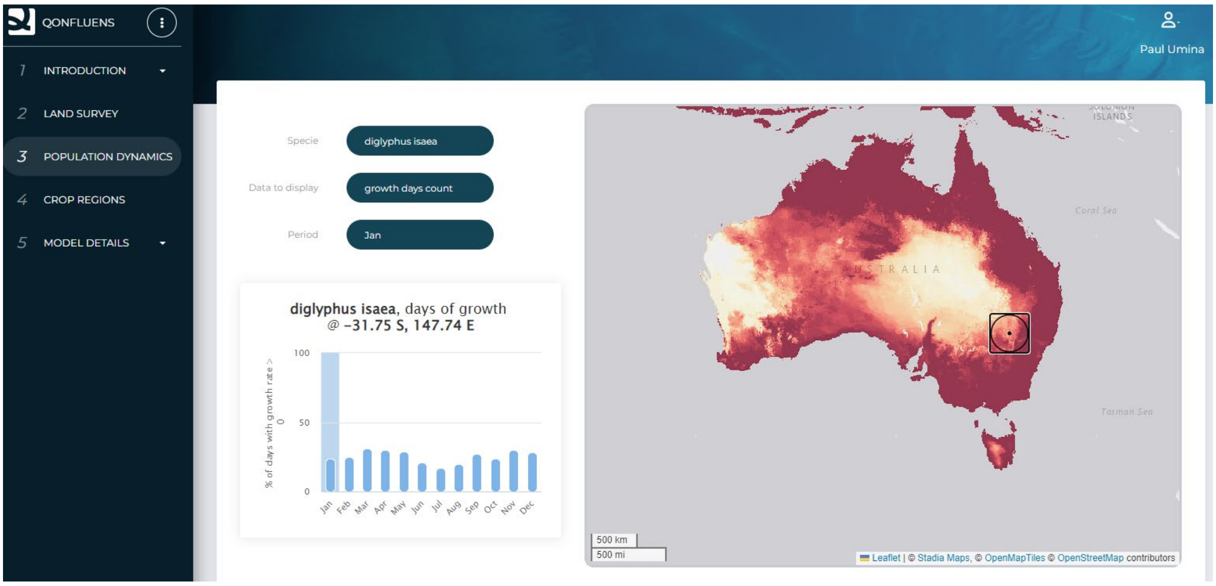


Figure 7: Screenshot of the Flask-VueJs app.This app requires to login.

References

Schouten R, Baudrot V, Umina P, Maino J (2022). A working guide to writing mechanistic spatial ecological models in Julia. *Methods in Ecology and Evolution* 13, 945-954.

Appendix 6.1

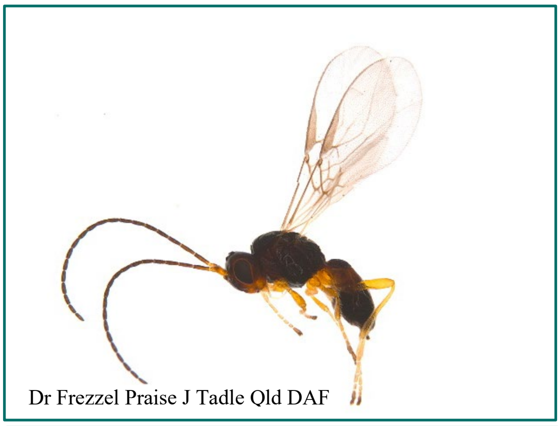
Identification of parasitoid wasps reared from *L. huidobrensis* and *L. trifolii*

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by project partners

P.M. Ridland, X. Xu and A.A. Hoffmann

Dr Frezzel Praise J Tadle Qld DAF

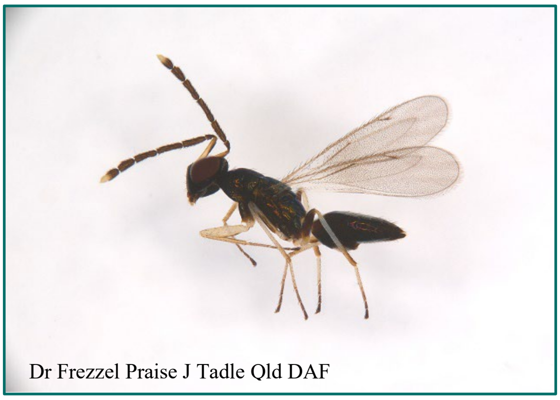


Dr Frezzel Praise J Tadle Qld DAF

Dr Andrew Manners Qld DAF

Dr Elia Pirtle Cesar Australia

Dr Frezzel Praise J Tadle Qld DAF



Dr Frezzel Praise J Tadle Qld DAF

Identification of parasitoid wasps reared from *L. huidobrensis* and *L. trifolii* by project partners.

PM Ridland, X. Xu and AA Hoffmann, Pest & Environmental Adaptation Research Group (PEARG), School of BioSciences, The University of Melbourne

Summary

A significant community of parasitoid wasps has been reared in each region from either *Liriomyza huidobrensis* or *L. trifolii*. However, species composition and complexity varied between regions. This probably reflects the fact that the diversity of parasitoid community changes in time as infestations of *Liriomyza* spp. spread. The primary challenge will be to ensure these parasitoids are not disrupted by excessive use of inappropriate insecticides and that reservoirs of parasitoids and non-target agromyzids are identified and managed/conserved effectively.

The parasitoid wasp community reared from *L. trifolii* in NW Western Australia (1,587 parasitoids reared from samples collected between April 2022 and November 2022) was dominated by the idiobiont ectoparasitoids *Zagrammosoma latilineatum* (57%) and *Hemiptarsenus varicornis* (41%). Five other parasitoid species were identified [*Asecodes* sp., *Apotosoma* sp., *Neochrysocharis formosa*, *N. okazakii* (Eulophidae); *?Gronotoma* sp. (Figitidae, Eucoilinae)] and together with some unidentified Pteromalidae were in very low numbers (2% of all parasitoids). Most of the mined leaves sampled were from the Asteraceae (65%) and Fabaceae (25%). There were no *Opius* spp. (Braconidae) reared from puparia. Within the Asteraceae, 91% of parasitoids were reared from sunflower. Peak numbers of *H. varicornis* and *Z. latilineatum* were recorded in August 2022. In the Northern Territory, the main parasitoids recorded from *L. trifolii* were *H. varicornis*, *Z. latilineatum* and *N. formosa*. An important finding was that *Opius* sp.1 (most likely part of the *O. atricornis* complex) has now been found in low numbers in the NT.

In SE Queensland, 16 identified parasitoid species plus some unidentified eulophids and pteromalids were reared from *L. huidobrensis* (1,279 parasitoids in all). The most abundant parasitoid species were *H. varicornis* (40% of all specimens), *Opius cinerariae* (23%), *N. formosa* (15%), *Asecodes* sp. (6%) and *N. okazakii* (4%). These were the first confirmed Queensland records of *Asecodes* sp., *N. formosa*, *N. okazakii* and *Diglyphus isaea* (all species have been previously recorded from SE Australia attacking agromyzids). The major differences between the parasitoid communities observed in NW WA and SE Qld were the relatively low abundance of *Z. latilineatum* and the high abundance of *O. cinerariae* in SE Qld. In NSW, six species (*D. isaea*, *H. varicornis*, *N. formosa*, *N. okazakii*, *O. cinerariae* and *?Gronotoma* sp.) were reared from *L. huidobrensis* mining faba bean. *Neochrysocharis formosa* was recorded for the first time in NSW and was the most abundant parasitoid.

Specimens of ?*Gronotoma* sp. (Figitidae: Eucoilinae) were reared from *L. huidobrensis* (NSW and Qld) and *L. trifolii* (WA and NT). These are the first Australian records of a eucoiline parasitizing an agromyzid. Specimens will be sent to USA for examination by a specialist in the family.

Introduction

Worldwide, the polyphagous *Liriomyza* leafminers are secondary pests with outbreaks occurring when (i) their suite of parasitoids becomes reduced in cropping systems by insecticides targeting leafminers or other pests in the crop, or (ii) naturally occurring parasitoids are not present in greenhouses (Ridland *et al.* 2020). The value of parasitoids in regulating populations of *Liriomyza* spp. has long been recognised. Secondary pest flare-ups following the disruption of parasitoid communities by insecticides have been documented frequently for *Liriomyza* spp. (Hills & Taylor 1951; Oatman & Kennedy 1976; Johnson *et al.* 1980a, 1980b, 1980c; Hidrayani *et al.* 2005; Chirinos *et al.* 2017).

Invading polyphagous *Liriomyza* spp. are usually attacked by a suite of generalist parasitoids already present in a country parasitising a range of other non-pestiferous leafmining species, including both agromyzids and lepidopteran leafminers (Murphy & LaSalle 1999; Reitz *et al.* 2013). Many parasitoids of agromyzids specialise in the leaf mine niche rather than being taxonomically restricted to a particular host species (Ubaidillah *et al.* 2000; Salvo *et al.* 2011).

In Australia, there is already a suite of generalist parasitoids that parasitize a range of agromyzid species, which should contribute to regulating exotic *Liriomyza* pests (Ridland *et al.* 2020). Agromyzid species colonizing weeds and non-crop plants serve as valuable reservoirs to support parasitoid wasps, potentially providing parasitoids for the biological control of invasive *Liriomyza* spp. (Lardner, 1991; Bjorksten *et al.* 2005; Lambkin *et al.* 2008; Wood *et al.* 2010; Ridland *et al*. 2020).

With larval parasitoids (idiobionts), the adult female wasps can kill as many host larvae by host-feeding as they do by parasitising host larvae (Cheng *et al.* 2017). Consequently, the larval parasitoids reduce fly numbers developing from the current generation, as well as subsequent generations of *Liriomyza* larvae.

For mass-release in protected cropping, European growers make three releases of idiobiont wasps such as *D. isaea* or *N. formosa*, one week apart (which is expensive). These parasitoids are not yet available commercially in Australia, although starter cultures of *D. isaea* have been supplied from our laboratory to Dan Papacek of Bugs for Bugs, Mundubbera Qld for testing.

Larval-pupal parasitoids (koinobionts) reduce the abundance of flies in the next generation. The parasitoid wasp does not paralyse the host larva before oviposition and the parasitoid larva does not develop in the host larva until after the host larva exits the mine. Both types of parasitoids complement each other well in the field, but only idiobiont wasps are suitable for augmentative release in protected cropping.

This project section aimed to quantify the species composition, phenology, and abundance of hymenopteran parasitoids of *L. trifolii* in NW WA and NT, and *L. huidobrensis* in NSW and SE Queensland.

Methods

Project entomologists collected the samples in each region following the agreed sampling protocols for structured crop surveys or for scouting surveys. Agromyzid flies and suspected hymenopteran parasitoids were reared from the sampled mined leaves and stored in absolute alcohol for identification. Suspected parasitoids were sent to the University of Melbourne for identification.

We received 233 samples putatively infested with parasitoids of *L. trifolii* from NW Western Australia and found parasitoids in 196 of these samples. We also processed 106 samples of parasitoid wasps reared primarily from *L. huidobrensis*, but also from *L. brassicae*, *Calycomyza humeralis*, *Phytomyza syngenesiae* and *Tropicomyia polyphyta* in SE Queensland (from 1,517 specimens in all). In addition, some samples were received for identification from New South Wales (NSW) and the Northern Territory (NT). Processing has included morphological identification, scanning electron micrographs (SEM) and molecular barcoding as needed.

Results

(i) Western Australia

The parasitoid community reared from *L. trifolii* in NW Western Australia (1,587 parasitoids reared from samples collected between April 2022 and November 2022) was dominated by *Zagrammosoma latilineatum* and *Hemiptarsenus varicornis*, both idiobiont ectoparasitoids (Table 1). The five other parasitoid species recorded [*Asecodes* sp., *Apotosoma* sp., *Neochrysocharis formosa* ♀, *Neochrysocharis okazakii* ♀ & ♂ (Eulophidae); *?Gronotoma* sp. (Figitidae, Eucoilinae)] were in very low numbers. There were no *Opius* spp. reared from puparia (Table 2). It will be interesting to see when an *Opius* sp. appears in the region, given that *Opius* sp.1 (most likely part of the *O. atricornis* complex) has now been found in low numbers in the

Northern Territory. In the Lesser Sunda Islands of Indonesia, the major parasitoids of *Liriomyza* spp. were *H. varicornis*, *N. formosa*, *N. okazakii*, *Asecodes deluchii*, *Opius dissitus*, *O. chromatomyiae* and *Gronotoma micromorpha* (Wahyuni *et al.* 2017). The major difference in community structure in NW WA with the Lesser Sundas was the abundance of *Z. latilineatum* and the absence of *Opius* spp. in NW WA. Based on the observations in SE Queensland where 16 identified species of hymenopteran parasitoids of *L. huidobrensis* were recorded in this project, it is likely that the diversity of parasitoid species will increase with time. Again, the major difference between the two states was the relatively low abundance of *Z. latilineatum* and the high abundance of *O. cinerariae* in SE Queensland.

Most of the mined leaves sampled were from Asteraceae (65%) and Fabaceae (25%), with very few observed on Brassicaceae, Cucurbitaceae, Malvaceae and Solanaceae (Table 3). Parasitoids were most abundant in Asteraceae (83% of total number reared) and Fabaceae (11%) (Table 4). Within the Asteraceae, 91% of parasitoids were reared from sunflower (Table 5). Peak numbers of parasitoids were reared in August 2022 for *H. varicornis* (Table 6) and *Z. latilineatum* (Table 7).

Inevitably, when mined leaves are collected for rearing parasitoids of agromyzids and then held in plastic bags for emergence, other arthropods collected, such as thrips, aphids, beetles and spiders. The conservative approach taken in WA was for all arthropods found in the emergence bags to be retained for checking. In the WA samples, thrips were the most common additional arthropod collected from the sampled leaves but were initially erroneously counted as parasitoids of *L. trifolii* (Asteraceae 63%; Fabaceae 84%) when sent for ID (Table 8). Hymenopteran parasitoids not attacking agromyzids but which sometimes emerged from sampled leaves included (i) mymarids, which often parasitize eggs of thrips or leafhoppers, (ii) aphidiid wasps, which emerge from mummified aphids and (iii) trichogrammatids emerging from lepidopteran eggs (Table 8).

Table 1 Dominant parasitoid species reared from *Liriomyza trifolii* in NW Western Australia

Parasitoid Species	**n**	**% of total**
Zagrammosoma latilineatum	907	57.2%
Hemiptarsenus varicornis	654	41.2%
All other parasitoid species	26	1.6%
	1,587	100.0%

Table 2 Parasitoid species (showing sexes) reared from *Liriomyza trifolii* in NW Western Australia (all identified species are eulophids, apart from one eucoiline)

Parasitoid Species	**n**	**% of total**
Zagrammosoma latilineatum ♀	543	34.2%
Zagrammosoma latilineatum ♂	364	22.9%
Hemiptarsenus varicornis ♂	345	21.7%
Hemiptarsenus varicornis ♀	309	19.5%
Asecodes sp.	4	0.3%
Neochrysocharis formosa ♀	2	0.1%
Neochrysocharis okazakii ♀	2	0.1%
Neochrysocharis okazakii ♂	1	0.1%
Apotosoma sp.	1	0.1%
?Gronotoma sp. (Figitidae: Eucoilinae)	1	0.1%
unidentified eulophids	11	0.7%
unidentified pteromalids	4	0.3%
∑	**1,587**	100.0%

Table 3 Numbers of mines found on sampled leaves from each plant family

Plant Family	∑ mines on sampled leaves*	%total
Asteraceae	6,023	66.4%
Fabaceae	2,241	24.7%
Malvaceae	310	3.4%
Cucurbitaceae	238	2.6%
Brassicaceae	131	1.4%
Solanaceae	115	1.3%
Amaranthaceae	14	0.2%
Passifloraceae	0**	-
Lamiaceae	0**	-
∑	**9,072**	

*

** No mines were recorded, but parasitoids were recorded

Table 4 Parasitoid species reared from *Liriomyza trifolii* from different plant families in NW Western Australia

Parasitoid	Asteraceae	Fabaceae	Malvaceae	Solanaceae	Brassicaceae	Cucurbitaceae	Passifloraceae	Lamiaceae	Grand Total
Zagrammosoma latilineatum	745	141	20	0	0	1	0	0	907
Hemiptarsenus varicornis	600	36	1	3	6	5	2	1	654
*Asecode*s	0	1	0	3	0	0	0	0	4
Neochrysocharis okazakii	2	0	0	1	0	0	0	0	3
Neochrysocharis formosa ♀	1	0	0	1	0	0	0	0	2
?*Gronotoma*	0	1	0	0	0	0	0	0	1
Apotosoma sp.	0	1	0	0	0	0	0	0	1
unidentified eulophid	11	0	0	0	0	0	0	0	**11**
unidentified pteromalid	4	0	0	0	0	0	0	0	**4**
∑	**1,363**	**180**	**21**	**8**	**6**	**6**	**2**	**1**	**1,587**
% of Grand Total	**85.3%**	**11.3%**	**1.3%**	**0.50%**	**0.38%**	**0.38%**	**0.13%**	**0.06%**	

Table 5 Parasitoid species reared from *Liriomyza trifolii* by plant family in NW Western Australia

Asteraceae	Sunflower	Safflower	Lettuce*	Marigold	Daisy weed	∑
Zagrammosoma latilineatum ♀	418	22	6	2	0	448
Hemiptarsenus varicornis ♂	286	12	12	2	3	315
Zagrammosoma latilineatum ♂	262	33	2	0	0	297
Hemiptarsenus varicornis ♀	263	10	8	2	2	285
Neochrysocharis okazakii ♂	1	0	0	0	0	1
Neochrysocharis formosa ♀	0	0	0	1	0	1
Neochrysocharis okazakii ♀	1	0	0	0	0	1
unidentified eulophid	9	0	2	0	0	11
unidentified pteromalid	4	0	0	0	0	4
∑	**1,244**	**77**	**30**	**7**	**5**	**1,363**
% of Asteraceae	**91.3%**	**5.6%**	**2.2%**	**0.5%**	**0.4%**	

* lettuce, Romain lettuce, leaf lettuce, iceberg lettuce

Fabaceae	Bean	Green bean	Snake bean	Snowpea	∑
Zagrammosoma latilineatum ♀	39	37	0	2	78
Zagrammosoma latilineatum ♂	39	24	0	0	63
Hemiptarsenus varicornis ♂	12	4	0	2	18
Hemiptarsenus varicornis ♀	13	2	0	3	18
Apotosoma sp.	0	1	0	0	1
?Gronotoma sp.	0	1	0	0	1
Asecodes sp.	0	0	1	0	1
∑	**103**	**69**	**1**	**7**	**180**

Malvaceae	Cotton
Zagrammosoma latilineatum ♀	16
Zagrammosoma latilineatum ♂	4
Hemiptarsenus varicornis ♀	1
∑	**21**

Solanaceae	Tomato
Asecodes sp.	3
Hemiptarsenus varicornis ♀	3
Neochrysocharis okazakii ♀	1
Neochrysocharis formosa ♀	1
∑	**8**

Cucurbitaceae	Apple cucumber	Lebanese cucumber	Watermelon	∑
Hemiptarsenus varicornis ♂	2	1	0	3
Hemiptarsenus varicornis ♀	0	0	2	2
Zagrammosoma latilineatum ♀	0	1	0	1
∑	**2**	**2**	**2**	**6**

Brassicaceae	Rocket
Hemiptarsenus varicornis ♂	6

Passifloraceae	Passionfruit vine
Hemiptarsenus varicornis ♂	2

Lamiaceae	***Salvia splendens*** **cv. Vista Purple**
Hemiptarsenus varicornis ♂	1

Table 6 Records of *Hemiptarsenus varicornis* reared from *Liriomyza trifolii* in NW Western Australia, by month, site and host plant

Hemiptarsenus varicornis	May	June	July	August	September	October	November	∑
Asteraceae		**9**	**56**	**476**	**52**		**7**	**600**
Broome				**5**				**5**
leaf lettuce				2				2
leaf lettuce				3				3
Broome -residential garden		**1**						**1**
iceberg lettuce		1						1
Ceres			**37**	**471**				**508**
sunflower			37	471				508
Frank Wise Institute (Kimberley Research Station)		**3**	**19**		**4**			**26**
marigold					4			4
marigold		3	19					22
Oria					**48**			**48**
lettuce					7			7
lettuce					41			41
Tanami Park		**5**						**5**
daisy weed?		5						5
Kununurra Riverfarm Rd							**7**	**7**
iceberg lettuce							7	7
Fabaceae		**26**	**8**			**2**		**36**
Barradale		**17**	**8**					**25**
bean		17	8					25
Broome						**2**		**2**
snowpea						2		2
Broome TAFE		**4**						**4**
green bean		1						1
snowpea		3						3
Ceres		**5**						**5**
green bean		5						5
Brassicaceae						**6**		**6**
Broome						**6**		**6**
rocket						6		6
Cucurbitaceae		**2**			**2**	**1**		**5**
Broome TAFE		**2**						**2**
watermelon		2						2

Hemiptarsenus varicornis	May	June	July	August	September	October	November	∑
Oria					**2**	**1**		**3**
apple cucumber					1	1		2
Lebanese cucumber					1			1
Solanaceae						**3**		**3**
Broome						**3**		**3**
tomato						3		3
Passifloraceae		**2**						**2**
Broome -residential garden		**2**						**2**
passionfruit vine		2						2
Malvaceae	**1**							**1**
Frank Wise Institute (Kimberley Research Station)	**1**							**1**
cotton	1							1
Lamiaceae		**1**						**1**
Broome TAFE		**1**						**1**
salvia cv Vista Purple		1						1
∑	**1**	**42**	**64**	**476**	**54**	**12**	**7**	**656**

Table 7 Records of *Zagrammosoma latilineatum* reared from *Liriomyza trifolii* in NW Western Australia, by month, site and host plant

Zagrammosoma latilineatum	May	June	July	August	September	October	∑
Asteraceae		**12**	**46**	**655**	**32**		**745**
Broome				**2**			**2**
leaf lettuce				2			2
Broome -residential garden		**2**					**2**
iceberg lettuce		2					2
Ceres			**5**	**649**	**2**		**656**
sunflower			5	649	2		656
Frank Wise Institute (Kimberley Research Station)		**10**	**41**	**4**	**2**		**57**
marigold					2		2
safflower		10	41	4			55
Oria					**28**		**28**
lettuce					4		4
sunflower					24		24
Fabaceae		**119**	**19**	**2**		**1**	**141**
Barradale		**56**	**19**	**2**			**77**

Zagrammosoma latilineatum	May	June	July	August	September	October	∑
bean		56	19	2			77
Broome Tafe		**4**					**4**
green bean		2					2
snowpea		2					2
Ceres		**59**					**59**
green bean		59					59
Wyndham						**1**	**1**
bean						1	1
Malvaceae	**20**						**20**
Frank Wise Institute (Kimberley Research Station)	**20**						**20**
cotton	20						20
Cucurbitaceae					**1**		**1**
Oria					**1**		**1**
Lebanese cucumber					1		1
∑	**20**	**131**	**65**	**657**	**33**	**1**	**907**

Table 8 Arthropods collected from WA emergence samples that are not parasitoids of *Liriomyza trifolii*

Specimen	**Asteraceae**	**Brassicaceae**	**Cucurbitaceae**	**Fabaceae**	**Malvaceae**	**Solanaceae**	**∑**
thrips	54			103	4	1	162
unidentified wasps	2		19	3		1	25
Liriomyza trifolii (teneral)	3			16			19
leafhopper	15				2	1	18
aphid	2	1					4
beetle	2			1			3
midge	2						2
agromyzid puparium			1				1
alate aphid	1						1
arthropod fragment	1						1
blackened puparium	1						1
bug	1						1
Collembola	1						1
lacewing larva						1	1
spider	1						1
unidentifiable fragment			1				1
∑	**86**	**1**	**21**	**123**	**6**	**4**	**241**

(ii) Queensland

There were 16 identified parasitoid species plus some unidentified eulophids and pteromalids reared from *L. huidobrensis* sampled in SE Queensland (1,279 parasitoids in all). The most abundant parasitoid species were *Hemiptarsenus varicornis* (40% of all specimens), *Opius cinerariae* (23%), *Neochrysocharis formosa* (15%), *Asecodes* sp. (6%) and *Neochrysocharis okazakii* (4%) (Table 10). Total abundance rankings for *O. cinerariae* and *N. formosa* were greatly influenced by very large records from one sample (101 and 147 parasitoids, respectively), which were from a heavily-infested sample from a celery crop residues after harvest. and represented only a subset of the parasitoids reared (Table 10). The frequency of occurrence of a particular species in specimens is a more useful statistic of relative abundance: *H. varicornis* (62 records from 90 samples of leaves infested with *L. huidobrensis*) and *O. cinerariae* (29 records) were the most frequently recorded species, in contrast, *N. formosa* was 4 x more abundant but occurred at a similar frequency to *N. okazakii* (17 records for each species).

The outlying sample highlighted the ability of populations of parasitoids to grow explosively when there is sufficient time and hosts for multiple generations to develop in the crop cycle (Table 10). From the sample of 22 heavily-mined celery leaves, 310 *L. huidobrensis* flies emerged from puparia, 400 larval-pupal parasitoids emerged from puparia, and 260 larval parasitoids emerged from the leaves. This observation highlights the potential use of augmentoria as a way of collecting parasitoids from mined foliage, while preventing adult *Liriomyza* flies from escaping into the crop (Ridland *et al.* 2020). Further studies should be undertaken.

The first confirmed Queensland records of *Asecodes* sp., *Neochrysocharis formosa*, *N. okazakii* and *Diglyphus isaea* were made (all species have been previously recorded from SE Australia attacking agromyzids). These four species are significant parasitoids of *Liriomyza* spp. overseas. Dr Christer Hansson, University of Lund, Sweden, has provided valuable taxonomic advice on some of the eulophid species we have identified. To assist the morphological identifications, SEM images have been taken of representative specimens and molecular barcoding also done for some of the specimens (Xu *et al.* 2023). These barcoding results add to our database of barcodes of parasitoids of leafminers and the international database more generally. We are working on the opportunity to use them in a metabarcoding exercise to allow for a high throughput of wasp sample screening.

Specimens of ?*Gronotoma* sp. (Figitidae: Eucoilinae) have been reared from *L. huidobrensis* (NSW & Qld) and *L. trifolii* (WA and NT). These records are the first Australian records of a figitid parasitizing an agromyzid. Specimens will be sent to the USA for examination by a specialist in the family. *Gronotoma* is a relatively common parasitoid of agromyzids in tropical areas (Wahyuni et al. 2017).
While only low numbers of *Diglyphus isaea* (2%) were found, molecular barcoding and SEM imaging have confirmed the presence of this important species in Queensland. Previously, *D. isaea* had only been recorded from New South Wales, Tasmania and Victoria (Ridland *et al.* 2020). SEM imaging for all species will prove very useful as a resource for identifying parasitoids of leafminers going forward. The composition of the parasitoid fauna will vary seasonally and spatially as the SLM and ASLM infestations continue to spread. Life history information about each species is summarized in Table 11.

Most parasitoids of *L. huidobrensis* were reared from mined celery (Table 12). Again, 16 parasitoid species were identified, with a few eulophids and pteromalids still needing to be identified.

Nine species of parasitoids were reared from *Phytomyza syngenesiae* mining sowthistle (*Sonchus oleraceus*) (180 parasitoids from 9 samples) in SE Queensland (Table 13). Again, in terms of frequency of occurrence, *H. varicornis* and *N. okazakii* were the most frequently recorded species (6 of 9 records).

Asecodes sp. was the most abundant parasitoid species (61 of 64 specimens) reared from the epidermal-mining agromyzid, *Tropicomyia polyphyta*, from kapok vine (*Araujia sericifera*) (from 2 samples) and native passionfruit (*Passiflora herbertiana*) (from 1 sample) in SE Queensland (Table 14). So far, very little is known about the life history of *Asecodes* sp. attacking agromyzids in Australia. Details of its morphology are given by Xu *et al.* (2023).

Endosymbiont screening of some Queensland parasitoid wasps revealed *N. formosa* specimens (only female specimens were observed, so thelytoky is suspected) were infected with *Rickettsia* bacterium, which is related to the *Rickettsia* present in thelytokous populations of *N. formosa* in Japan, China and Victoria (Xu *et* *al.* 2022). Intriguingly, we also found two endosymbionts (*Rickettsia* and *Wolbachia*) co-infecting individuals of *N. okazakii* (without a sign of thelytoky), suggesting that the *Wolbachia* infection has suppressed the impact of *Rickettsia* on thelytoky. These findings provide background information about the parasitoid fauna expected to help control the leafminers into the future. Mass-rearing would benefit greatly if the generation of parthenogenetic lines of parasitoids for release can be achieved (to avoid the issue of male bias in reared stock).

Table 10 Hymenopteran parasitoids reared from *Liriomyza huidobrensis** in SE Queensland (samples collected between June 2021 and January 2023)

Parasitoid species	Parasitoid Family	∑	% of total	Max.*	No. of records
Hemiptarsenus varicornis	Eulophidae	511	39.8%	40	62
Opius cinerariae	Braconidae	299	23.3%	101**	29
Neochrysocharis formosa	Eulophidae	197	15.3%	147**	17
Asecodes sp.	Eulophidae	73	5.7%	28	12
Neochrysocharis okazakii	Eulophidae	47	3.7%	11	17
?*Gronotoma* sp.	Figitidae	39	3.0%	13	11
Opius sp. 1	Braconidae	32	2.5%	9	12
Diglyphus isaea	Eulophidae	28	2.2%	9	11
Zagrammosoma latilineatum	Eulophidae	8	0.6%	2	7
Atoposoma sp.	Eulophidae	4	0.3%	4	1
Trigonogastrella parasitica	Pteromalidae	3	0.2%	1	3
Vagus ambiguus	Eulophidae	2	0.2%	2	2
Pediobius sp.	Eulophidae	2	0.2%	1	2
Aprostocetus sp.	Eulophidae	1	0.1%	1	1
Apleurotropis sp.	Eulophidae	1	0.1%	1	1
Sphegigaster sp.	Pteromalidae	1	0.1%	1	1
eulophid unidentified	Eulophidae	7	0.5%	1	7
pteromalid unidentified	Pteromalidae	24	1.9%	4	17
	∑	**1,279**			

*includes mixed populations of *Liriomyza huidobrensis/L. brassicae, L. huidobrensis/L. chenopodii, L. huidobrensis/Calycomyza humeralis*, where both species were recorded from particular hosts but specific identification of agromyzid species present was not always carried out.

** Atypical sample from heavily-infested celery left well after harvest (usually would have been slashed and ploughed in). In this sample, 22 leaves were collected, yielding 260 parasitoids from the leaves (174 of these parasitoids were sent for ID i.e. 67%); 1286 puparia were collected, yielding 310 *L. huidobrensis* adults and 400 parasitoids, i.e. 55% emergence (101 of these parasitoids [all *O. cinerariae*] were sent for ID, i.e. 25%)

Table 11 Life history strategies of hymenopteran parasitoids reared from *Liriomyza huidobrensis** in SE Queensland (samples collected between June 2021 and January 2023)

Parasitoid Type	**Mode of Action**	**Effect on Host Larva**	**Family**	**Species**	**No**	**%**	**Comments**
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Hemiptarsenus varicornis*	511	39.8%	Most common species at the start of the Qld incursion
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Braconidae	*Opius cinerariae*	299	23.3%	An Australian species frequently reared from agromyzids in eastern Australia
endoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Neochrysocharis formosa*	197	15.3%	A female-only species, with potential for mass-rearing for use in protected cropping
endoparasitoid	larval (idiobiont) but needs confirmation	?paralyse	Eulophidae	*Asecodes* sp.	73	5.7%	Very small wasp. Further work required on its biology
endoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Neochrysocharis okazakii*	47	3.7%	Both sexes found, unlike *N. formosa*
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Figitidae	?*Gronotoma* sp.	39	3.0%	First Australian record parasitising agromyzids
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Braconidae	*Opius* sp. 1	32	2.5%	
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Diglyphus isaea*	28	2.2%	potential for mass-rearing for use in protected cropping
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Zagrammosoma latilineatum*	8	0.6%	This species was commonly reared from *L. sativae* at Seisia, far NQ [E. Pirtle unpublished data].
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Atoposoma* sp.	4	0.3%	
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Pteromalidae	*Trigonogastrella parasitica*	3	0.3%	
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Vagus ambiguus*	2	0.2%	
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Pediobius* sp.	2	0.2%	
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Aprostocetus* sp.	1	0.1%	
ectoparasitoid	larval (idiobiont)	paralyse	Eulophidae	*Apleurotropis* sp.	1	0.1%	
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Pteromalidae	*Sphegigaster* sp.	1	0.1%	
?	?larval (idiobiont)	?paralyse	Eulophidae	eulophid unidentified	7	0.5%	
endoparasitoid	larval-pupal (koinobiont)	non-paralyse	Pteromalidae	pteromalid unidentifed	24	2.1%	
				∑∑	**1,279**		

*includes mixed populations of *Liriomyza huidobrensis/L. brassicae, L. huidobrensis/L. chenopodii, L. huidobrensis/Calycomyza humeralis*, where both species were recorded from particular hosts but specific identification of agromyzid species present was not always carried out.

Table 12 Parasitoids reared from *Liriomyza huidobrensis* from celery (*Sonchus oleraceus*) (from 9 samples) in SE Queensland

Parasitoid species		∑parasitoids	Max. no. parasitoids*	No. of records
Hemiptarsenus varicornis	Eulophidae	444	40	52
Opius cinerariae	Braconidae	292	101**	26
Neochrysocharis okazakii	Eulophidae	42	11	15
Neochrysocharis formosa	Eulophidae	191	147**	14
Asecodes sp.	Eulophidae	72	28	11
?Gronotoma sp.	Figitidae	29	13	7
Diglyphus isaea	Eulophidae	24	9	9
Opius sp. 1	Braconidae	22	9	10
Zagrammosoma latilineatum	Eulophidae	8	2	7
Atoposoma sp.	Eulophidae	5	5	1
Trigonogastrella parasitica	Pteromalidae	3	1	3
Vagus ambiguus	Eulophidae	2	1	2
Pediobius sp.	Eulophidae	1	1	1
Aprostocetus sp.	Eulophidae	1	1	1
Apleurotropis sp.	Eulophidae	1	1	1
Sphegigaster sp.	Pteromalidae	1	1	1
eulophid unidentified	Eulophidae	4	1	4
pteromalid unidentified	Pteromalidae	23	4	14
		1,165		

*Maximum no. of parasitoids recorded in a sample
** Atypical sample from heavily-infested celery left well after harvest (typically would have been slashed and ploughed in). In this sample, 22 leaves were collected, yielding 260 parasitoids from the leaves (174 of these parasitoids were sent for ID i.e. 67%); 1286 puparia were collected, yielding 310 *L. huidobrensis* adults and 400 parasitoids, i.e. 55% emergence (101 of these parasitoids [all *O. cinerariae*] were sent for ID, i.e. 25%)

Table 13 Parasitoids reared from *Phytomyza syngenesiae* from sowthistle (*Sonchus oleraceus*) (from 9 samples) in SE Queensland

Parasitoid species	**Parasitoid family**	**∑**	**Max. no.***	**No. of records**
Asecodes sp.	Eulophidae	42	29	3
Neochrysocharis okazakii	Eulophidae	34	7	6
Hemiptarsenus varicornis	Eulophidae	32	10	6
Neochrysocharis formosa	Eulophidae	30	17	3
Zagrammosoma latilineatum	Eulophidae	16	12	5
Opius cinerariae	Braconidae	11	5	2
Opius sp. 1	Braconidae	2	7	1
Diglyphus isaea	Eulophidae	1	1	1
?*Gronotoma* sp.	Figitidae	1	1	1
eulophid unidentified	Eulophidae	8	1	2
pteromalid unidentified	Pteromalidae	3	1	1
∑		**180**		

*Maximum no. of parasitoids recorded in a sample

Table 14 Parasitoids reared from *Tropicomyia polyphyta* from kapok vine (*Araujia sericifera*) (from 2 samples) and native passionfruit (*Passiflora herbertiana*) (from 1 sample) in SE Queensland

Parasitoid species	Kapok vine	Native passionfruit	∑
Asecodes sp.	51	14	65
Apleurotropis sp.		1	1
Chrysocharis sp.	1		1
Opius sp. 2	1		1
∑	**53**	**15**	**68**

(iii) New South Wales

Most of the reared parasitoids were identified in NSW, but some specimens were sent to the University of Melbourne for identification.

Six species were recorded from faba bean from *L. huidobrensis.* Seven species of parasitoid wasps were recorded from mined leaves on sowthistle. It is highly probable that the host agromyzid on sowthistle was *Phytomyza syngenesiae*. (Table 15). *Neochrysocharis formosa* was recorded for the first time in NSW and was the most abundant parasitoid.

Table 15 Parasitoids recorded from a range of agromyzids in the Sydney Basin and subsequently identified at The University of Melbourne

Parasitoids	Faba bean	Sowthistle	Spinach
Asecodes sp.		✓	
Chrysocharis pubicornis		✓	
Diglyphus isaea	✓	✓	
Hemiptarsenus varicornis	✓	✓	
Neochrysocharis formosa	✓		
Neochrysocharis okazakii	✓		
Zagrammosoma latilineatum		✓	
Opius cinerariae	✓	✓	
?Gronotoma (Figitidae)	✓	✓	
Trigonogastrella parasitica			✓

(iv) Northern Territory

In the NT, the main parasitoids recorded from *L. trifolii* were *H. varicornis*, *Z. latilineatum*, *N. formosa* and *Vagus ambiguus*. An important finding was that *Opius* sp.1 (most likely part of the *O. atricornis* complex) has now been found in low numbers in the NT.

Conclusions and Recommendations for Future Research

Findings from the Project

1. A significant community of parasitoid wasps have been reared in each region from either *L. huidobrensis* or *L. trifolii*. However, the species composition and complexity varied between regions. The numbers of species recorded is likely to increase in time.
2. Parasitoid wasps should play an important role in controlling *Liriomyza* spp. in Australia, provided they are managed correctly, particularly in relation to use and timing of appropriate insecticides. As a rule of thumb, the longer a crop is in the ground before harvest, then the greater the control of *Liriomyza* spp. will be. Very short-rotation crops such as spinach and leafy greens will be unlikely to derive much direct benefit from parasitoids, since the crop will be harvested before the parasitoids can develop a second generation. However, high parasitism in other crops should reduce the invasion pressure of *Liriomyza* flies for the new crops.
3. Thelytokous (female-only) populations of *Neochrysocharis formosa* were found in each region. Populations in Queensland were infected with the same *Rickettsia* bacterium found in thelytokous

populations of *N. formosa* in Japan, China and Victoria. There will be value in further studies of the impact on endosymbiont infection on the reproductive biology of parasitoid species. Production of female-only lines of parasitoids will improve the economics of mass-rearing parasitoids for augmentative biocontrol.

Further parasitoid studies needed

4. Improved taxonomic resolution of parasitoids of Australian agromyzids likely to attack *Liriomyza* spp. is required, along with the development of molecular markers for rapid identification of parasitoids. These tools will greatly assist researchers assessing the impact of endemic parasitoids. There would be great value in developing metabarcoding approaches to allow for a high throughput of wasp sample screening.
5. There is a need to clarify the taxonomy and biology of the complex of opiine braconid parasitoids already found attacking agromyzids in Australia. Genetic evidence is emerging that several species complexes are present. There is an urgent need to redescribe *Opius atricornis* and *Opius oleracei*. The holotypes and allotypes of both these species (and 18 paratypes of *O. atricornis*) are held at the Queensland Museum.
6. Studies are needed on the biology, ecology and distribution of parasitoids in Australia, such as *Diglyphus isaea*, *Neochrysocharis formosa*, *N. okazakii*, *Zagrammosoma latilineatum*, *Closterocerus mirabilis*, *Asecodes* sp., *Proacrias* sp. (Family Eulophidae), *Trigonogastrella parasitica* (Family Pteromalidae; Sub-family Pteromalinae), and *Opius cinerariae* (Family Braconidae; Sub-family Opiinae) to assess their potential role in controlling *L. huidobrensis*, *L. sativae* and *L. trifolii* in open-air production as well as their potential suitability for use in augmentative biological control of the three species in protected cropping.

Augmentative release of parasitoids

7. Mass-produced parasitic wasps are very expensive to produce in large quantities. They are only suitable for protected cropping because multiple releases of idiobiont wasps such as *Diglyphus isaea* and *Hemiptarsenus varicornis* are needed early in the crop to ensure substantial levels of host-killing as well as parasitism. Koinobiont species such as *Opius* spp. do not host feed and parasitised *Liriomyza* larvae continue to mine leaves. The koinobiont parasitoid does not begin to develop until the parasitised *Liriomyza* larva exits the mine and pupates. Releasing *Opius* spp. in protected cropping would only be worthwhile for long-lived crops (where multiple generations of *Liriomyza* occur) and situations where *Liriomyza* spp. overwinter in greenhouses.
8. *Diglyphus isaea* is the predominant species released worldwide, largely because it has relatively high fecundity. While some attempts to mass-rear *H. varicornis* and *N. formosa* have been made overseas, these species are seldom mass-reared.
9. While mortality caused by host feeding by the idiobiont wasps is a very important factor for growers, biological control agent (BCA) producers need to maximise the number of female wasps produced from a given batch of host *Liriomyza* larvae. Any male wasps produced are of little use to growers since they do not paralyse larvae – they host-feed on larvae paralysed by female wasps.
10. There is a great advantage for BCA producers and growers to have female-only lines, since every wasp sold and released will be targeting *Liriomyza* larvae. In Australia, we have already identified three eulophid species parasitising agromyzids that appear to be thelytokous: *Neochrysocharis formosa*, *Proacrias* sp. and *Aprostocetus* sp. All three species are infected with the endosymbiont *Rickettsia*. However, none of these species has a fecundity to match *D. isaea*. Ideally, a female-only line of *D. isaea* would be the ideal candidate for mass-rearing as costs would be reduced for growers.
11. There is a need to check populations throughout Australia to see if any thelytokous populations of *D. isaea* or *H. varicornis* are present.
12. The second approach would be to transfer the *Rickettsia* into Australian cultures of *D. isaea*. Transfer of endosymbionts using microinjection is being undertaken on a range of species in the Hoffmann lab. If successful, endosymbiont transfer to create female-only strains of natural enemies would be of great benefit to a range of crops, not just involving leafminers.
13. Producing parasitic wasps is only the first step. Detailed studies would be needed to evaluate the impact of augmentative releases. Successful biological control in glasshouses will also need very careful selection of control methods for other pests of the crop in question.

14. It is also vital to develop appropriate quality control procedures to ensure that mass-reared lines continue to perform appropriately.

Conservation biological control

15. The primary challenge will be to ensure these parasitoids are not disrupted by excessive use of inappropriate insecticides and that the reservoirs of parasitoids and non-target agromyzids are identified and managed effectively.
16. Movement patterns of parasitoids from reservoirs of non-crop plants to crops are unknown, as is the dispersal of agromyzids and their parasitoids through wider cropping areas, adjoining non-crop areas and sequentially planted crops.
17. The use of augmentoria as a way of collecting parasitoids from mined foliage, while preventing adult *Liriomyza* flies from escaping into the crop, should be investigated.
18. Understanding the impact of parasitoids and other natural enemies on *Liriomyza* spp. would benefit greatly from life table and natural exclusion studies such as conducted successfully for *Plutella xylostella* in SE Queensland.

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Xu X, Hoffmann AA, Umina PA, Coquilleau MP, Gill A & Ridland PM. 2022. Identification of two leafminer parasitoids (Hymenoptera: Eulophidae), *Neochrysocharis formosa* and *Proacrias* sp. from Australia, with both showing thelytoky and infection by *Rickettsia*. *Austral Entomology* **61**, 358–369. https://doi.org/10.1111/aen.12602 [Open Access]

Appendix

Two recent scientific papers from the Pest & Environmental Adaptation Research Group (PEARG), The University of Melbourne, cover morphometric identification, molecular characterization and endosymbiont status of a range of adventive and native hymenopteran parasitoids of agromyzids from Australia. The most abundant parasitoids reared from *L. huidobrensis* and *L. trifolii* in Australia are covered in these studies.

Xu X, Hoffmann AA, Umina PA, Coquilleau MP, Gill A & Ridland PM. 2022. Identification of two leafminer parasitoids (Hymenoptera: Eulophidae), *Neochrysocharis formosa* and *Proacrias* sp. from Australia, with both showing thelytoky and infection by *Rickettsia*. *Austral Entomology* **61**, 358–369. https://doi.org/10.1111/aen.12602 [Open Access]

Abstract:
Liriomyza huidobrensis, *L. sativae* and *L. trifolii* are polyphagous agromyzid leafminers that have recently arrived in Australia, posing a threat to Australian vegetable and ornamental crops. Adventive and endemic hymenopteran parasitoids of agromyzid leafminers already present in Australia should assist in the management of these invasive agromyzid species. *Neochrysocharis formosa* (Hymenoptera: Eulophidae: Entedoninae) is an idiobiont endoparasitoid commonly attacking *Liriomyza* spp. in many countries, but it has not been formally identified in Australia. This study provides the first confirmed Australian record of *N. formosa* as well as an unidentified *Proacrias* species, another entedonine species. Females of both species were reared from several adventive and endemic agromyzid leafminers in southern Australia. Laboratory cultures of both species established the presence of thelytokous reproduction. DNA barcodes (5′ COI, 3′ COI, ITS1, ITS2, 28S) were used to delineate species boundaries, with the 5′ end of the mitochondrial COI sequences pointing to multiple cryptic lineages among *N. formosa*. Additionally, 16S rRNA sequencing indicated that both species were infected with a *Rickettsia* bacterium, which is related to the *Rickettsia* present in thelytokous populations of *N. formosa* in Japan and China. These findings expand records for parasitoids attacking leafminers in Australia and highlight the potential for an endosymbiont to produce thelytokous strains that could provide for more efficient biocontrol agents for augmentative release.

Xu X, Hoffmann AA, Umina PA, Ward SE, Coquilleau MP, Malipatil MB & Ridland, PM. 2023. Molecular identification of hymenopteran parasitoids and their endosymbionts from agromyzids. *Bulletin of Entomological Research,* **113**, 481–496. https://doi.org/10.1017/S0007485323000160
[Open Access]

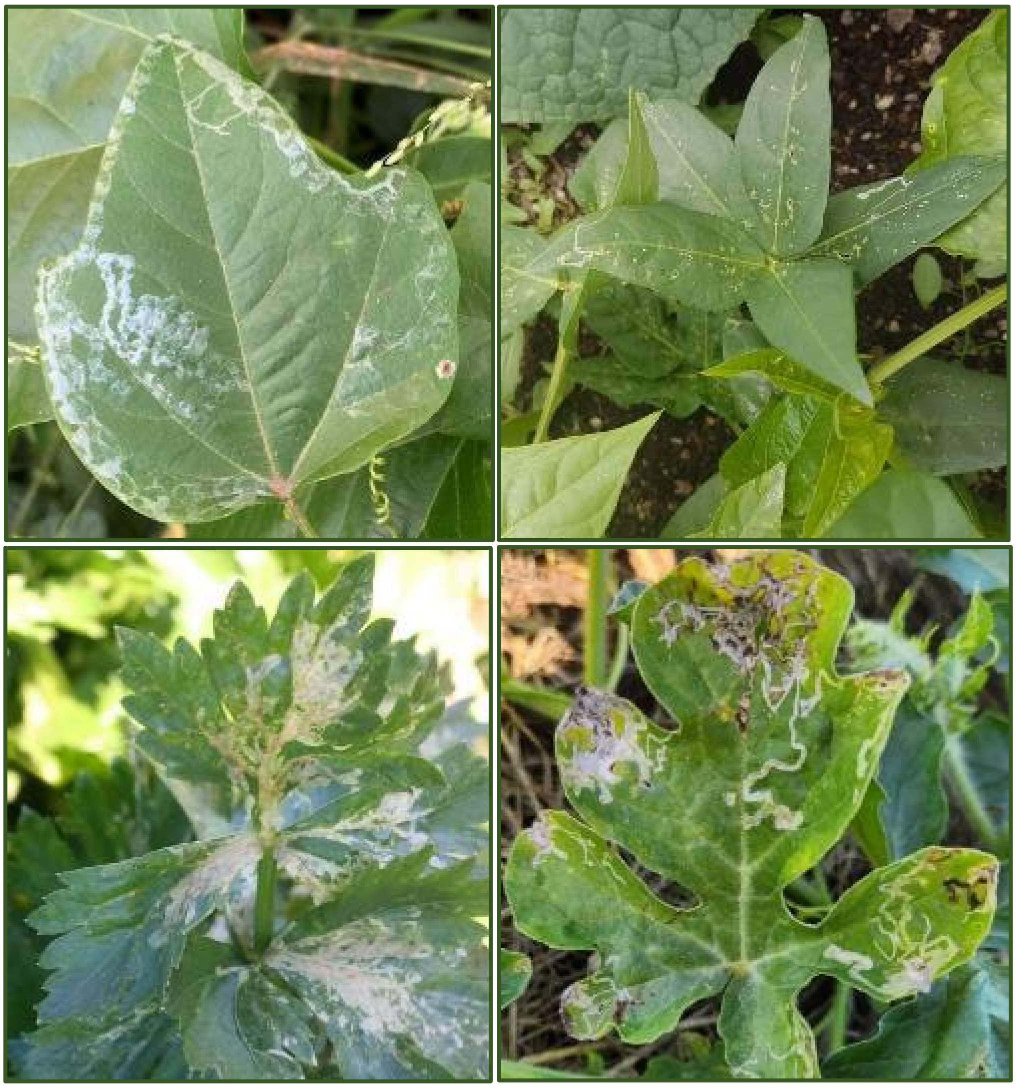
Abstract: Three polyphagous pest *Liriomyza* spp. (Diptera: Agromyzidae) have recently invaded Australia and are damaging horticultural crops. Parasitic wasps are recognized as effective natural enemies of leafmining species globally and are expected to become important biocontrol agents in Australia. However, the hymenopteran parasitoid complex of agromyzids in Australia is poorly known and its use hindered due to taxonomic challenges when based on morphological characters. Here, we identified 14 parasitoid species of leafminers based on molecular and morphological data. We linked DNA barcodes (5′ end *cytochrome c oxidase subunit I* (COI) sequences) to five adventive eulophid wasp species (*Chrysocharis pubicornis* (Zetterstedt), *Diglyphus isaea* (Walker), *Hemiptarsenus varicornis*(Girault), *Neochrysocharis formosa* (Westwood), and *Neochrysocharis okazakii* Kamijo) and two braconid species (*Dacnusa areolaris* (Nees) and *Opius cinerariae* Fischer). We also provide the first DNA barcodes (5′ end COI sequences) with linked morphological characters for seven wasp species, with three identified to species level (*Closterocerus mirabilis* Edwards & La Salle, *Trigonogastrella parasitica* (Girault), and *Zagrammosoma latilineatum* Ubaidillah) and four identified to genus (*Aprostocetus* sp., *Asecodes* sp., *Opius* sp. 1, and *Opius* sp. 2). Phylogenetic analyses suggest *C. pubicornis*, *D. isaea*, *H. varicornis,* and *O. cinerariae* are likely cryptic species complexes. *Neochrysocharis formosa* and *Aprostocetus* sp. specimens were infected with *Rickettsia*. Five other species (*Cl. mirabilis*, *D. isaea, H. varicornis, Opius* sp. 1, and *Opius* sp. 2) were infected with *Wolbachia*, while two endosymbionts (*Rickettsia* and *Wolbachia*) co-infected *N. okazakii.* These findings provide background information about the parasitoid fauna expected to help control the leafminers.

Appendix 7.1

Crop protection gap analysis, including how controlling Serpentine, American serpentine and Vegetable leafminers will fit into current management strategies and resistance

MT20005 Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

by John D Duff



Crop protection gap analysis, including how controlling Serpentine, American serpentine and Vegetable leafminers will fit into current management strategies and resistance.

Key gaps remaining:
As this project progresses, a crop protection gap analysis will be conducted to identify where additional R&D support may be required. Some initial areas where gaps should be identified include adequate coverage of affected crops with appropriate chemical permits; information necessary for area wide management approaches that account for all pests and chemical uses in a system to preserve beneficial insects; availability of economic thresholds and monitoring protocols for population size estimation.

Gaps in our knowledge

When leafminers were first detected on the mainland of Australia, in particular *Liriomyza huidobrensis*, growers cried out for insecticides to manage them. This was particularly the case with leafy vegetables, where the quality of the harvestable product was severely affected. Crops were ploughed in as with certain baby leaf vegetables, or infested leaves trimmed off to try and minimise the incidence of leafminer evidence on the crop such as wombok, silverbeet and celery.

It is therefore fitting to start this gap analysis off with a look at insecticides, what is currently available for use and what is on the horizon and just why are they needed.

Just because an insecticide if registered for use against leafminers, doesn't mean that it will be effective. Not all leafminers are flies, some are beetles (lantana leaf mining beetle - *Octotoma scabripennis*) while others are moths (citrus leafminer - *Phyllocnistis citrella*; potato/tomato leafminer -*Phthorimaea operculella*). The correct identification is crucial so that the correct insecticide can be applied to give maximum control so long as it is also applied in the correct manner, which we will discuss latter.

Insecticide use
Since the introduction of *Liriomyza sativae* (VLM) onto Torres Strait Islands in 2008 and then 2015 on the mainland of Cape York Peninsula, and the more recent introductions of *L. huidobrensis* (SLM) in 2020 and *L. trifolii* (ASLM) in 2021, the horticultural industry has managed to successfully apply for 11 permits as shown in the table below. There are an additional 4 minor use permits available for the nursery industry. They all have contact action and some systemic activity, either translaminar or systemically moved around the plant via the xylem (chlorantraniliprole) or phloem (thiamethoxam) or both as with spirotetramat. Getting the product to where it is needed, in the leaf is vital, if leafminers are going to be managed with IPM friendly insecticides. The use of harsh insecticides such as synthetic pyrethroids and organophosphates have limited systemic capabilities if any and are known to kill off a wide range of insects, both pests and beneficials alike. They are predominantly contact insecticides with a number being highly residual, so still affecting ALL insects, days after they have been applied to the crop.

There is a need to seek registration of certain products for leafminer flies, as permits only last so long, with the last insecticide permit expiring on the 30 June 2024. Those insecticides currently with permits, there is a need to include leafminer flies on their labels. So lobbying the various chemical companies to seek an expansion of labels need to be undertaken. With

the majority already registered for us overseas, the APVMA may be able to fast track this process.
Investigating the options for use of new products that are coming on to the market could help growers strategically use insecticides as part of an IPM program, while favouring the build of beneficial insects. Even having such insecticides as azadirachtin become available for use could help growers better manage this pest. This product is used overseas, including New Zealand, with hopes that it will become available for use within Australia.

Other insecticides that could be of use:
Sivanto Prime - flupyradifurone (FPF) from Bayer (contact)
Simodis – isocycloseram from Syngenta (contact)
Azamax – azadirachtin from Organic Crop Protectants (Weintraub and Horowitz, 1997) (limited systemic activity)
SeroX – *Clitoria ternatea* extract (anti-feedant)
Trebon – etofenpox (Al-Kazafy et al., 2015)

However, insecticide use has been shown to be the least beneficial for the management of leafminer flies due primarily to the disruption of beneficial insect populations.

Reitz et al, (2013) documented information on the intense insecticide use being the most common strategy used to eradicate newly discovered outbreaks of *Liriomyza* spp. (Bartlett and Powell, 1981). The success of this strategy is dependent on the susceptibility of invasive population to available insecticides. Because invasive populations are already likely to be resistant to various insecticides (MacDonald, 1991), eradication programs may not be successful. Understanding the levels of resistance that invasive species bring in with them will help growers better manage their leafminer situation. **So testing recently arrived exotic populations needs to be undertaken on a wider range of insecticides with known resistance overseas.**

Table 1. Pesticide permits currently available for the fruit and vegetable industries.

Permit No.	Active	Crop/s	Pest	Jurisdiction	Issued	Expiry	Permit Holder
PER81867 Version 2	Cyromazine (Diptex 150 WP)	Broccoli Fruiting vegetables – cucurbits, Fruiting vegetables – other than cucurbits (excluding mushrooms and corn), Head lettuce, Legume vegetables, Root and tuber vegetables, Stalk and stem vegetables (Field and Protected Cropping) **Use on the following crops is not supported for food use:** Brassicas (excluding Broccoli) Leafy vegetables (excluding Head lettuce) (Field and Protected Cropping) **These crops must be destroyed if treated and must not be made available for human consumption**	Liriomyza species, including: Vegetable Leafminer (Liriomyza sativa) Serpentine Leafminer (Liriomyza huidobrensis	All States and Territories	2-Dec-19	30-Nov-23	Hort Innovation
PER81876 Version 4	Abamectin (Various 18g/L & 36 g/L)	Fruiting vegetables – cucurbits, Fruiting vegetables – other than cucurbits (except sweet corn and mushrooms), Leafy vegetables (except lettuce), Legume vegetables, Root and tuber vegetables, Bulb onions, Cabbage (head), Celery and Rhubarb Bulb Vegetables except Bulb onions (including leeks, spring onions)	Leaf miners (Liriomyza spp.) Suppression only Including Vegetable Leafminer (Liriomyza sativae) and Serpentine Leafminer (Liriomyza huidobrensis)	ALL States except Vic	24-Jun-16	30-Apr-24	Hort Innovation
PER87631 Version 2	Chlorantraniliprole Coragen plus other 200 g/L registered	Spinach and Silverbeet	Leaf miners (Liriomyza spp.) including: Cabbage leafminer (Liriomyza brassicae) Vegetable leafminer (Liriomyza sativae) Serpentine leafminer (Liriomyza huidobrensis) (suppression only)	All States and Territories except Vic	21-Jun-19	30-Jun-24	Hort Innovation
PER90387	Cyantraniliprole	As per the Benevia label Bulb vegetables, Fruiting vegetables (all) Potatoes	Leaf miners (Liriomyza spp.) Including: Vegetable leaf miner (Liriomyza sativae) Pea leafminer/Serpentine leafminer (Liriomyza huidobrensis) American serpentine leafminer (Liriomyza trifolii)	All States and Territories except Vic	3-Dec-20	31-Dec-23	Hort Innovation
PER87563	Emamectin (Various 17g/L & 44g/kg products)	BRASSICA VEGETABLES (including broccoli, brussels sprouts, cabbage, cauliflower)	LEAFMINERS (Liriomyza species) Including Vegetable Leafminer (Liriomyza sativae) Suppression Only	All States & Territories except Vic	6-Jun-19	30-Jun-24	Hort Innovation

PER87878 Version 2	Spinetoram (Success Neo & Delegate only)	Snow Peas Sugar Snap Peas Green beans	Liriomyza Leafminers (Liriomyza spp.)	All States and Territories except Vic	11-Feb-20	28-Feb-23	Hort Innovation
PER91155	Spinetoram (Success Neo)	Brassica vegetables; including Broccoli Brussels sprouts Cabbage Cauliflower Brassica leafy vegetables Cucurbits; including Cucumber, Melon, Squash, Zucchini, Culinary Herbs Fruiting vegetables; including Eggplant, Okra, Peppers (Capsicums & Chillies) Sweet corn, Tomato Leafy vegetables; including Lettuce, Endive, Silverbeet, Spinach & Brassica leafy vegetables Root and tuber vegetables; including Beetroot, Carrot, Celeriac, Galangal, Parsnip Potato, Radish (incl. Daikon), Sweet potato, Swede, Stalk & Stem vegetables; including Celery Rhubarb	Leaf miners (Liriomyza spp.) Including: Vegetable leaf miner (Liriomyza sativae) Pea leaf miner/Serpentine leaf miner (Liriomyza huidobrensis) American serpentine leaf miner (Liriomyza trifolii)	All States and Territories except Vic	9-Jun-21	30-Jun-24	Hort Innovation
PER88640	Spirotetramat (Movento 240 SC)	Snow Peas, Sugar Snap Peas, Lettuce (Head lettuce and Leafy lettuce), Parsley, Eggplant, Capsicums, Chilies, Tomatoes (Field and protected cropping systems) Green Beans, Celery, Rhubarb (Field cropping systems)	Liriomyza leafminers (Liriomyza spp.) (including Vegetable leafminers, Pea leafminer and American serpentine leafminer) Suppression only	All States and Territories except Vic	18-May-20	31-May-23	Hort Innovation
PER90928	Spinosad (Entrust Organic Insecticide)	Cucurbits including cucumber, melon, squash, zucchini Culinary Herbs Fruiting vegetables Leafy vegetables including, Lettuce, Endive, Silverbeet, Spinach & Brassica leafy vegetables Legume vegetables (succulent seeds and immature pods only); including bean pea snow pea & sugar snap pea Root and tuber vegetables; including Beetroot, Carrot, Celeriac, Galangal Parsnip, Potato Radish (incl. Daikon) Sweet potato, Swede, Turnip Stalk & Stem vegetables; including Celery Rhubarb *ORNAMENTALS - Nursery (Non-Bearing)*	Leafminers (*Liriomyza* spp.) Including: Vegetable leafminer (*Liriomyza sativae*) Pea leaf miner/Serpentine leafminer (*Liriomyza huidobrensis*) American serpentine leafminer (*Liriomyza trifolii*)	All States and Territories except Vic	23-Apr-21	30-Apr-24	Hort Innovation
PER90927	Cyantraniliprole (Benevia Insecticide)	Celery	Leafminers (*Liriomyza* spp.) Including: Vegetable leafminer (*Liriomyza sativae*) Pea leafminer/Serpentine leafminer (*Liriomyza huidobrensis*) American serpentine leafminer (*Liriomyza trifolii*)	All States and Territories except Vic	5-May-21	31-Dec-23	Hort Innovation
PER91161	Chlorantraniliprole + Thiamethoxam (Durivo Insecticide)	Brassica leafy vegetables Including: Chinese broccoli, Chinese cabbage, Garden cress, Kale and Rocket Leafy vegetables Including: Lettuce, Endive, Silverbeet, Spinach, Chard	Leaf miners (Liriomyza spp.) Including: Vegetable leafminer (Liriomyza sativae) Pea leafminer/Serpentine leafminer (Liriomyza huidobrensis) American serpentine leafminer (Liriomyza trifolii)	All States and Territories except Vic	9-Jun-21	30-Jun-24	Hort Innovation

Table 2. Pesticide permits currently available for the nursery industry.

Permit No.	Description	Issued date	Expiry date	Permit Holder
PER83506	CYROMAZINE / NURSERY STOCK / Larvae of: Leafminer flies and Sciarid flies Including, Fungus gnats and Shore flies	24-Oct-17	31-Oct-22	Nursery & Garden Industry Australia
PER88977	Various Products; Abamectin, Azadirachtin, Cyromazine, Emamectin, Chlorantraniliprole + Thiamethoxam (Durivo), Cyantraniliprole, Indoxacarb, Spinetoram / Nursery Stock / Leafminers (*Liriomyza* spp.) including Vegetable leafminer (*Liriomyza sativae*)	11-Nov-20	30-Nov-22	Nursery & Garden Industry Australia
PER88695	Abamectin (Voliam Targo Insecticide) / Various Nursery Stock / Various Pests including Leafminer (*Liriomyza sativae*)	8-Feb-21	28-Feb-24	Greenlife Industry Australia Ltd
PER89239	Cyclaniliprole (Teppan Insecticide) / Nursery Stock (Non-food) / Various Pests including Leafminers (*Liriomyza spp*.)	5-May-21	31-May-23	Greenlife Industry Australia Ltd

Chemical baseline data on insecticide resistance introduced with the exotic leafminers, and development of resistance management strategies will be crucial in trying to understand the management of these leafminer flies. Understanding the insecticide resistance status of the Australian populations of VLM, SLM and ASLM will be key to ongoing management. There is a need to generate this baseline chemical sensitivity data for key insecticides which will be needed to monitor for future resistance evolution in the field. In the absence of established genetic diagnostics, the most likely approach to determine resistance is to conduct laboratory phenotypic bioassays. Resistance status should also be used to inform the creation of resistance management strategies that rely upon mode of action rotations and IPM practices. SLM and ASLM are being raised in laboratory colonies in QLD and NSW for the SLM and the NT for ASLM. Unfortunately, VLM is not being raised in the laboratory and so the availability of suitable specimens for testing is limited to when surveys are undertaken on the Cape York Peninsula where this species is currently restricted to. There is also a risk that this species could be displaced by the ASLM which is also present in the same area, making it harder to identify a pure colony of VLM for testing purposes. Trying to establish a colony in far north QLD could be problematic due to the biosecurity issues and availability of personnel to look after and maintain such a colony solely for insecticide resistance testing.

Gaps in knowledge

Newer effective insecticides and how they affect parasitoids, also what effect those under permit do to parasitoids.
Resistance levels to insecticides of introduced Liriomyza populations
Availability of VLM for resistance testing
Where did the exotic species originate from

Insecticide Application

The effectiveness of any insecticide is partly due to how it is applied to the crop and where in the crop you need to get the insecticide to. Current application methods include overhead spray boom, either air assisted or conventional spray boom without air. Controlled droplet application or CDA is also an option for some growers. Spray coverage is a major issue, trying to get chemicals to where the pests are causing the greatest amount of damage. Leafminers are particularly troublesome, as they are most often found attacking older leaves. In crops such as celery and silverbeet, getting sprays to the older lower leaves is always going to be a challenge due to the nature of growing the crops close together. Markets demand crops be grown in specific ways such as tall bunches of celery with less bushing and leaf material lower down which is why they are now planted close together. Something that now needs looking into due to the nature of leafminers attacking such closely planted crops. A rethink on just what growers can get away with while still producing a saleable product needs to be addresses by both growers and their markets.

Is there a better way to get the insecticides to where they are needed the most (drench, trickle or even reducing the planting density). Some pesticides are better taken up through the roots so there is a need to look at the efficacy of different application approaches for a range of insecticides such as Group 4 and 28 insecticides. How much and how often to apply such insecticides. The insecticide azadirachtin gave better control of SLM if applied to the roots rather than the leaves (Weintraub and Horowitz, 1997, Costa et al., 2018) and against VLM in trials on tomatoes in Thailand resulting in the death of 100% of first instar larvae (Hossain et al., 2008). There was some effect of a soil applied application of Spinosad to developing larvae in bean plants (Weintraub and Mujica, 2006).

Incorporating the use of a penetrating surfactant improves management with lower rates of insecticides, this approach may also help reduce selection pressures and it is reasonable that increasing penetration of abamectin or cyromazine into plants would likewise increase their efficacy (Reitz et al., 2013). Etofenprox is a systemic insecticide and can be absorbed by the roots and the leaves and transmitted to the plant tissues (Al-Kazafy et al., 2015).

Encapsulation technology of insecticides where the insecticide is released slowly for the plant to uptake (Hack et al., 2012, Perlatti et al., 2013). Does this give a greater window of protection or does it compromise MRL values and therefore the withholding periods? There is a lot of information out there on types of encapsulation, and this may only be a desk top study at this stage to see if it is possible.

Gaps in knowledge
Efficacy of different application methods
Encapsulation technology
Planting densities. Would increasing plant spacing improve spray coverage and control.

Integrated Crop Management approach
Integrated Pest Management IPM or more accurately, Integrated Crop Management ICM, is the bringing together of a range of tools or practices designed to alleviate the impact that a pest or disease has on the growth of a crop or cropping system. This has traditionally involved the use of biological control, cultural and physical control options and the strategic use of pesticides, which is considered the option of last resort, not the first.

An ICM approach can involve as little a crop monitoring to inform the grower just what is present in the crop, both pest and beneficial, to a full blown approach to changing growing practices to combat the pest or disease, selection of resistant varieties, the use of biological control agents and then the use of pesticides to help supplement the control of the pest or disease being targeted.

Leafminer ICM in Australia is in its very early days, with chemical choice being the top of the list of tools that growers are using. A number of these insecticides are however soft on beneficial insects, which is a step in the right direction. Other control options being investigated or may have an impact on leafminer activity involve biological control agents, crop monitoring, especially early in the crop and season, seasonal abundance of the various *Liriomyza* species, crop nutrition and crop hygiene practices.

Effective crop monitoring also includes the accurate identification of these leafminers as there are a number of similar looking native species out there such as *Lirioymza brassicae* or cabbage leafminer. This project has been able to develop molecular diagnostic techniques including qPCR and LAMP which would be best suited to research groups or diagnosticians within state agricultural departments or private research organisations. Whether these would be used by local agronomists would be doubtful due to the cost of individual units and the subsequent cost of tests. Is there an easier way to identify the various Liriomyza leafminers or is it necessary when your crop is being attacked by a leafminer affecting the harvestable crop and local knowledge is enough to recognise what leafminer it is. A better understanding of morphological characteristics might be more beneficial to agronomists and so could be collated into a special document (Maharjan et al., 2014, Weintraub et al., 2017, Mhatre et al., 2022, Chang et al., 2020).

ICM demonstration trials using all methods of control options (Chemical selection, biological control options, and cultural control options). These need to be undertake in key growing regions with leafminer infestations at the time of the year that is suitable for leafminer activity.

Management at the nursery level using parasitoids and a drench at dispatch using appropriate insecticide(s). Helping nurseries monitor and manage leafminers using ICM. How to send samples off for molecular ID, eDNA/LAMP.

Crop varieties could play a part in an ICM program. Why are some plants affected by leafminers and not others. Look at a range of varieties of certain crops that are known hosts of the various leafminers to see if there is any varieties out there that are not as heavily infested as others, growing them in leafminer hot spots in different growing regions.

Use of parasitoids – Best candidates for mass releases (*Hemiptarsenis, Opius, Diglyphus*) Discussions with biological service providers and how they can be developed for mass releases is essential.

The following sections are directly from the final report of MT16004 RD&E program for control, eradication and preparedness for vegetable leafminer, and cover areas that are gaps in our knowledge of Liriomyza leafminers and where additional funding could be directed.

1. *Augmentative release of parasitoids*
   Mass-produced parasitic wasps are very expensive to produce in large quantities. They are only suitable for protected cropping because multiple releases of ectoparasitoid wasps such as *Diglyphus isaea* and *Hemiptarsenus varicornis* are needed early in the crop to ensure substantial levels of host-killing as well as parasitism. Koinobiont species such as *Opius* spp. do not host feed and parasitised *Liriomyza* larvae continue to mine leaves. The koinobiont parasitoid does not begin to develop until the parasitised *Liriomyza* larva exits the mine and pupates. Releasing *Opius* spp. in protected cropping would only be worthwhile for long-lived crops (where multiple generations of *Liriomyza* occur) and situations where *Liriomyza* spp. were overwintering in the greenhouses.

   Diglyphus isaea is the predominant species released worldwide, largely because it has relatively high fecundity. While some attempts to mass-rear *H. varicornis* and *N. formosa* have been made overseas, these species are seldom mass-reared.

While mortality caused by host feeding by the idiobiont wasps is a very important factor for growers, BCA producers need to maximise the number of female wasps produced from a given batch of host *Liriomyza* larvae. Any male wasps produced are of little use to growers since they do not paralyse larvae – they host-feed on larvae paralysed by female wasps.

There is therefore a great advantage for BCA producers and growers to have female-only lines, since every wasp sold and released will be targeting *Liriomyza* larvae. In Australia, we have already identified three eulophid species parasitising agromyzids that appear to be thelytokous, a type of parthenogenesis: *Neochrysocharis formosa*, *Proacrias* sp. and *Aprostocetus* sp. All three species are infected with the endosymbiont *Rickettsia*. However, none of these species has a fecundity to match *D. isaea*. Ideally, a female-only line of *D. isaea* would be the ideal candidate for mass-rearing as costs would be reduced for growers.

Producing parasitic wasps is only the first step. Detailed studies would be needed to evaluate the impact of augmentative releases. Successful biological control in glasshouses will also need very careful selection of control methods for other pests of the crop in question.

It is also vital to develop appropriate quality control procedures to ensure that mass-reared lines continue to perform appropriately.

2. *Taxonomy and biology of parasitoids*

Improving the taxonomic resolution of parasitoids of Australian agromyzids likely to attack *Liriomyza* spp. is required, along with the development of molecular markers for rapid identification of parasitoids. These tools will greatly assist researchers assessing the impact of endemic parasitoids. There would be great value in developing metabarcoding approaches to allow for a high throughput of wasp sample screening. We have the approach running in our lab for other insects but it needs fine tuning for application to leafminer parasitoids.

Clarifying the taxonomy and biology of the complex of opiine braconid parasitoids already found attacking agromyzids in Australia. There is genetic evidence emerging that several species complexes are present. There is an urgent need to redescribe *Opius atricornis* and *Opius cinerariae*. Types of both these species are held at the Queensland Museum.

Studying the biology, ecology and distribution of parasitoids in Australia, such as *Diglyphus isaea*, *Neochrysocharis formosa*, *Neochrysocharis okazakii*, *Zagrammosoma latilineatum*, *Closterocerus mirabilis*, *Asecodes* sp., *Proacrias* sp. (Family Eulophidae), *Trigonogastrella parasitica* (Family Pteromalidae; Sub-family Pteromalinae), and *Opius cinerariae* (Family Braconidae; Sub-family Opiinae) to assess their potential role in controlling *L. huidobrensis*, *L. sativae* and *L. trifolii* in open-air production as well as their potential suitability for use in augmentative biological control of the three species in protected cropping.

3. *Endosymbionts*

Use of endosymbionts to produce female only offspring (Cesar – PhD candidate) This is Blue Sky thinking research.

There is a need to check parasitoid populations throughout Australia to see if any thelytokous populations of *D. isaea* or *H. varicornis* are present. So far only small samples have been screened.

A second approach would be to transfer the endosymbiont *Rickettsia* into Australian cultures of *D. isaea*. Transfer of endosymbionts using microinjection is being undertaken on a range of species in University of Melbourne lab. If successful, endosymbiont transfer to create female-only strains of natural enemies would be of great benefit to a range of crops, not just involving leafminers.

4. *Use of Entomopathogens*
   Are there any entomopathogenic fungi, bacteria, viruses or nematodes that could be utilised as control options. A literature review on the topic and possible future direction. (Gathage et al., 2016, Migiro et al., 2010)

   Entomopathogenic nematodes (Head et al., 2000, Williams and Macdonald, 1995) Nematodes are applied to leaves as a spray application and gain entry into the leaves via the feeding punctures caused by the female *Liriomyza* spp. Very high humidity is required for survival and efficacy of the nematodes which restricts their usefulness.

   Entomopathogenic fungi: Fungi is applied as a foliar spray (Devkota et al., 2016), in an autodissemination device (Migiro et al., 2010, Migiro et al., 2011, Gathage et al., 2016) or as an endophytic treatment where germinating seeds are colonised by fungal endophytes (including the entomopathogenic fungi, *Beauveria bassiana*) before planting (Akutse et al., 2013, Gathage et al., 2016). Preliminary field trials with strains of two endophytic fungi, *B. bassiana* and *Hypocrea lixii*, together with *Metarhizium anisopliae* applied in an autodissemination treatment gave promising results in terms of increased yields of common bean, Phaseolus vulgaris (Gathage et al. 2016).
   These methods require further development before being viable for commercial use.

5. *Plant extracts/attractants/pheromones*
   Attractants for use with yellow sticky traps eg aromatic oils (basil, clove, juniper etc)(Rizvi et al., 2015, Niu et al., 2022, Górski, 2005) have been shown to attract and trap more leafminer flies with between 100 and 500% increase in flies trapped. They may help to reduce resident populations but can they be used to manage infestations and damage on crops.

6. *Economic thresholds of various crops (potatoes, leafy veg, celery)*
   Not all crops are harvested for their foliage with most crops being able to withstand a percentage of damage to the foliage before there is a visible reduction in yield. This needs to be investigated for various crops that fall within this category such as potatoes, green beans and onions, just to name a few. Trials looking at various levels of damage or foliage removal will help determine just how much leaf material is required before there is a reduction in yield. Development of economic thresholds and validation of monitoring methods (such as sticky traps and pupal trays): Thresholds for pupa counts within trays, and sticky traps within field crops and closed cropping have not been developed within Australia for exotic Liriomyza pests. These techniques should be validated for SLM, ASLM and VLM and thresholds developed. Existing work on parasitoid biology and population modelling could be extended to develop thresholds for chemical pest

management (i.e. when parasitoids are present, are they insufficient for pest control and when do pesticide applications become warranted).

Gaps in our knowledge
Simple key to morphological characteristics for growers and agronomists
Augmentative release of parasitoids
Taxonomy and biology of parasitoids
Endosymbionts
Use of Entomopathogens
Plant extracts/attractants/pheromones
Economic thresholds of various crops (potatoes, leafy veg, celery)

Surveillance

This current project has looked at limited locations for leafminer activity. Surveys of new locations including nurseries across QLD, NSW, VIC, NT, WA (Cesar or NSW molecular group to help in ID's). Look at seasonal fluctuations of populations and spread of all 3 Liriomyza species from where they were first detected.

Using both LAMP and rapid infield diagnostics for rapid identification in key locations (From MT20005) In-field DNA extraction, eDNA with Cesar.
Ground truthing the findings from our current project in different locations with known infestations of leafminers.

Extension

Extension of findings using the Ausveg website, only as a conduit for disseminating information.
This is where they currently house all the leafminer information. Extension activities eg grower field days, workshops etc. Workshops along the lines of "Insect and Beneficial identification workshops" with emphasis on leafminers and fall armyworm and depending on what other exotic insect pests arrive into Australia, these could be include if they attack vegetable crops and nursery plants including vegetable seedlings. Information also needs to be added to the nursery technical website as nursery growers don't necessarily access the Ausveg website.

Collaboration

AS20002 Field trial evaluating insecticide resistance in serpentine leafminer (*Liriomyza huidobrensis*) and extension.

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Common leafminers identified as part of Project MT20005

Management strategy for serpentine leafminer, *Liriomyza huidobrensis*

Compiled by John D. Duff Qld DAF

American serpentine leafminer (ASLM) *Liriomyza trifolii*

L. trifolii has a vast host range and has been recorded from about 50 genera from 29 families with preference shown for the Asteraceae. Plants include vegetables and ornamentals.

Image: Central Science Laboratory, York (GB), British Crown

Serpentine leafminer (SLM) *Liriomyza huidobrensis*

L. huidobrensis is highly polyphagous and has been recorded worldwide from 365 host plant species in 49 plant families include beans, peas, beet, spinach, potatoes, tomatoes and cut flowers (including gypsophila, carnations and chrysanthemum).

Image: Central Science Laboratory, York (GB) , British Crown

Vegetable leafminer (VLM) *Liriomyza sativae*

L. sativae is a polyphagous pest of many vegetable and flower crops. It has been recorded from nine plant families, although its preferred hosts tend to be in the Cucurbitaceae, Fabaceae and Solanaceae

Image: Dr Elia Pirtle

Cabbage leafminer
Liriomyza brassicae

L. brassicae has been recorded from 16 genera in the Brassicaceae, Capparaceae, Resedaceae and Tropaeolaceae. It has also infrequently been recorded in the Fabaceae [Pisum and Lathyrus odoratus .

Image: Dr Elia Pirtle

Beet leafminer
Liriomyza chenopodii

This species is known only from genera from two closely related plant families: Caryophyllaceae (Cerastium, Silene, Stellaria) and Chenopodiaceae (Beta, Chenopodium, Spinaca)

Image: L. Semeraro

Tomato leafminer
Liriomyza bryoniae

L. bryoniae is highly polyphagous and has been recorded from 16 plant families. It is an important pest of tomatoes, cucurbits (particularly melons, watermelon and cucumber) and glasshouse-grown lettuce and beans.

Not yet present in Australia

Image: Koppert Global

Bean fly
Ophiomyia phaseoli

Bean fly is a pest of several summer pulses (including mungbean, navy beans, and black gram, but not soybean), particularly in coastal regions. Phasey bean (*Macroptillum lathyroides*) is a common weed host in agricultural areas, and other legume weeds may also be potential hosts. Bean fly is usually a pest of seeding crops, but infestations can also occur in later crop stages.

Image: Hugh Brier Qld DAF

Aster leafminer
Calycomyza humeralis

Found attacking fleabane weed and makes elongated blotch mines in many genera of the composites (Asteraceae) family.

Image: Ole Bidstrup

Lantana leafminer
Calycomyza lantanae

Biocontrol agent for lantana

Image: Melissa Duron 2019, Texas

Ragwort leafminer
Phytomyza syngenesiae

It is a polyphagous leaf miner that is mainly found on herbaceous Compositae (daisy family). It occurs in crops and, ornamental plants, as well as on native plants especially in the genus Senecio

Image: Tim Holmes Plant and Food Reseaerch

Plantain leafminer
Phytomyza plantaginis

The larva of the fly *P. plantaginis* mines the leaves of various Plantain species including Ribwort Plantain producing a long, linear, whitish narrow mine; normally in the leaf, but sometimes in the stem. The pupa is formed at the end of the mine and in the lower surface of the leaf.

Image: Rui Andrade iNaturalistUK

Pea leafminer
Chromatomyia horticola

C. horticola is a very serious pest, especially on glasshouse crops of tomatoes and lettuce. Infestations on seedlings are especially serious as the plants quickly lose the ability to develop, and wilt or die. Infestations on older plants can be less serious in effect, but yields are often reduced. Is recorded in around 268 genera of 36 families, commonly Brassicaceae, Fabaceae and Asteraceae
Not yet present in Australia
Image: Alchetron 2023

Chloropidae
Frit fly or grass fly

Many larvae are saprophages living in rotting or dying wood, usually in association with other insects, and in dead parts of herbaceous plants damaged by other insects. More rarely they feed in fungi. In a small number of species the larvae are predators and live in the egg cocoons of spiders, praying mantis, or the nests of locusts. Some species prey on root grubs.