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1 Acknowledgments

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2 Executive summary

The fall armyworm (FAW) *Spodoptera frugiperda* has caused major economic and social impact across Africa, Asia, and Oceania since its initial reported 2016 outbreak. The project (CROP/2020/144) aims to explore management options involving insecticides, IPM strategies, and cultural practices in Southeast Asia (SEA), and to understand and compare its population genetic diversity in SEA and Australia. By understanding genetic diversity, especially diversity in pesticide resistances, we aim to contribute to strategic management plans tailored for SEA and Australia farmers.

Whole genome sequencing of FAW from SEA (Myanmar, Vietnam, Lao PDR, Malaysia, Philippines), East Asia (South Korea) and Pacific (Papua New Guinea) showed significant genomic diversity between populations. This was reflected also in Australia's (i.e., Oceania) FAW populations due to Australia's close geographic proximity to SEA. FAW populations from SEA, East Asia, Pacific/Oceania, Africa. and other Asia regions all showed signatures of admixture, supporting the invasive populations as overwhelmingly belonging to hybrids of R- and C-strains. Effort to define invasive FAW based on host-preferences should therefore be discouraged as this is unlikely to help to predict host preference in the invasive range. A general lack of genetic connectivity between the early-stage northern Australia's FAW populations suggested multiple introduction pathways to Australia. The Myanmar and Yunnan Province (China) FAW populations exhibited different genomic signatures, and further supported its spread as multi-directional, with the establishment of invasive FAW populations likely underpinned by multiple independent introduction events, contradicting its unidirectional west-African origin and 'rapid' spread hypothesis.

The FAW genome resources generated from this project will assist with understanding of future gene flow between different FAW populations. It will also contribute to future development and implementation of national and regional management strategies especially relating to economically (e.g., insecticide resistance, allelochemical detoxification) and environmentally (e.g., relating to climate stressor adaptation, heat and drought tolerance, low temperature tolerance) important genetic traits. To this end, a simple metabarcoding approach was developed to demonstrate concurrent surveys of genetic diversity (relating to C- or R-strain haplotypes), and proportions of organophosphate/carbamate resistance allele frequencies from field FAW populations. This approach can be modified to track genes of interests with the support of the FAW genome resources. Better understanding of FAW migration patterns through global gene flow analyses will be needed, to inform how the FAW should be managed at national and regional levels. At regional and country levels, agricultural biosecurity preparedness (i.e., monitoring for FAW individuals with novel genome signatures) should be a priority to delay/minimise the introduction of new resistance genes into established populations. This project highlighted the global implication of pest incursions at local scales, especially for highly mobile species such as the FAW. Asia and SEA FAW likely contributed to establishment of Australia and some East African populations. With Australia's greater scientific capability, assistance to ASEAN communities to bolstering regional agricultural biosecurity could be a cost-effective solution to increase Australia's own national biosecurity preparedness against exotic and emerging agricultural pests and diseases.

The project provided opportunities for establishing a well-coordinated training and undertaking of insecticide and Bt bioassay experiments between Australia and SEA, and enabled the degree of tolerance/resistance to selected pesticides and Bt toxins to be quantified. The results suggested populations from different geographic regions responded differently to insecticides. While FAW are generally less sensitive to Cry1Ac and Cry2Ab toxins, the Indonesian population showed increased sensitivity to Cry1Ac as compared to Australia and Vietnam FAW. Western Australia and Indonesia FAW populations also showed highest indoxacarb tolerance than other SEA populations tested. Movements of these populations to other SEA regions are currently unknown, and could impact on regional management strategies.

The project explored cultural management practices undertaken by SEA countries and how these compared with East Africa. Similar approaches between Uganda and various SEA countries were reported, including intercropping, regular weeding/field sanitation, crop rotation, use of biological control/parasitoids, and using of trap crops/plants. The use of mechanical means (e.g., manually destroy egg masses) to manage the FAW were widely practiced. Progress was made to educate farmers through government/NGOs/inter-government-led awareness campaigns. Novel IPM and cultural pest management practices were developed in some SEA countries, i.e., using flooding to kill pupae, and the use of botanical extracts as spray additives. Despite these progresses, knowledge gaps and inconsistencies in IPM/cultural management practice advice between countries were identified. While the initial FAW outbreak caused anxiety among small-scale farmers, international collaborative effort to educate and help farmers to implement management strategies are giving back confidence and building resilience to the future management of this pest.

3 Background

Native to the Americas, *Spodoptera frugiperda* (fall armyworm, FAW) is a moth pest species consisted of two morphologically indistinguishable C- and R-strains (previously 'corn-preferred' and 'rice-preferred', respectively; see Tay et al. 2023) that was reported in Southeast Asia (SEA) since 2008 (Vu 2008), Asia/SEA since at least 2014 (Gilligan and Posada 2014), China since 2016 (Tay and Gordon 2019), and western and eastern Africa from 2016 (Georgen et al. 2016; Otim et al. 2018). Initially, maize yield losses of 20-50% were observed in African farms (Early et al. 2018). Serious damage has also been seen on e.g., ginger, sugarcane, sorghum from across African and Asian invasive ranges, and in its native New World range also in beet, tomato, potato, and cotton. Since the reported outbreak in Africa in 2016, the pest was confirmed also from Yemen, India, Sri Lanka, Bangladesh, Nepal, China, Myanmar, Vietnam, Thailand, Cambodia, Malaysia, Indonesia, and Laos PDR between May 2018 and December 2019. In January 2020, individuals were recorded in the Torres Straight islands of Saibai and Erub, followed by detection on mainland Australia (QLD, NT, WA, and NSW). Whilst armyworm pests are not new to cropping systems around the world, the FAW does pose a serious challenge to smallholder farmers in terms of sustainable management practices. Initial damage can look alarming and cause panic, however, several management options could be implemented. For example, a recent study in Zimbabwe showed that FAW damage was reduced in farms with frequent weeding operations and minimum- or zero-tillage (Baudron et al. 2019). The proximity of trees may support birds and insects that prey on the caterpillars. Importantly, inappropriate use of pesticides during a time of crisis can be avoided with adequate planning and preparation. The development of locally-specific integrated management options involving natural enemies and cultural control options, along with the careful use of insecticides, is required for areas where this pest has now established.

Attempts to distinguish between the C- and R-strains of FAW have relied on molecular markers including the maternally inherited mitochondrial DNA cytochrome oxidase subunit I (mt*COI*) or the sex-linked triosephosphate isomerase (*TPI*) gene on the Z chromosome. Hybrids of C- and R-strains FAW are known in native range populations (reviewed in Tay et al. 2023), but are especially prevalent in the invasive range populations (e.g., Tay et al. 2022a; Rane et al. 2022a; Jiang et al. 2022). Understanding the strain identity in invasive FAW populations has been viewed as necessary to assist with understanding host plant preferences (but see Tay et al. 2023) and to underpin development of management strategies of pest populations, although the two marker systems do not often agree in their strain diagnoses. This is due to the non-recombinant and uniparental (i.e., maternal) inheritance nature of the mitochondrial DNA genome, as well as the hemizygous nature of the Z chromosome in female Lepidoptera (i.e., ZW) as compared with male Lepidoptera (i.e., ZZ). While the *TPI* gene enables hybrids of C- and R-strain males to be identified, in females this often resulted in unreliable strain and hybrid status delineation, including for offspring of hybrid females (Tay et al. 2023; Juarez et al. 2014).

A further negative output of applying short partial single gene markers in understanding the invasive biology of FAW can be seen from studies that utilised the partial mt*COI* gene as population genetic marker. While the partial mt*COI* gene has been crucial in confirming the FAW in western Africa (Georgen et al. 2016), the highly conserved nature of this partial gene region could lead to misinterpretation that introduction of this pest involved single female founder (e.g., Nagoshi et al. 2017). Indeed, understanding of the pest's invasion biology at the early stages especially in Africa (and persisted to even now) has been that the pest first arrived in western Africa involving a single or very limited number of female founders, with subsequent detections being the results of rapid west-to-east spread. The persistent acceptance of the African origin and east-ward spread pattern involving single or limited founders could significantly and negatively impact on the development of management strategies for this pest, since on-going introductions of this pest from the native ranges to other invasive ranges would not be realised, and could lead to the introduction of novel undesirable genetic traits such as new climate adaptation or insecticide resistance genes.

While there had been various early research into crop host preferences by the C- and the R-strains FAW leading to the perception of host-plant preferences (i.e., C-strain preferring corn/maize, cotton and sorghum; R-strain preferring pasture grasses and rice), a recent comprehensive literature review (Tay et al. 2023) has found weak evidence of specific crop-host preferences between the two strains. Whilst the significance of these two strains for management remained unclear especially in their native ranges, certainly in the invasive range the need to distinguish between the C- and R-strains is becoming less important due to widespread presence of hybrid populations (e.g., Tay et al. 2022a; Rane et al. 2022a), although being able to distinguish novel C- or R-strain mitochondrial genomes would assist with monitoring of novel and potentially on-going introductions, including to assist with greater ease of detecting novel resistance genes. Detection of resistance genes is an essential activity in the management of FAW populations since this pest is well-known for its ability to evolve resistances to commonly used insecticides and in the Americas, including resistance to both conventional insecticides and to *Bt* in transgenic corn (Carvalho et al. 2013, Fatoretto et al. 2017, Banerjee et al. 2017, Flagel et al. 2018). For Australian cotton producers that rely on *Bt* transgenic technology and grain producers that use conventional pesticides, the incursion of this species with unknown resistance status, represents a significant threat to current IPM practices and the sustainability of the transgene technology. The genetic characterisation of populations that is present in Australia and SEA, and an understanding of whether known resistance traits are present in these populations are needed in the short-term.

Each SEA country and the Pacific/Australia is at a different stage in terms of their response to this new pest, and the research needs to be targeted in each case. In SEA several organisations (e.g. FAO, CABI, local plant protection institutes, Universities, government departments, ASEAN FAW Action Plan) are working in various partnerships to develop training packages for farmers, implement policy responses and characterise likely management options in the short-term. However, there are gaps in knowledge that are crucial to supporting future development activities and long-term management strategies. To that end we are addressing three research questions in this project:

1. What FAW management options exist and can be easily implemented in Southeast Asia (SEA)?
2. Which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management?
3. Do the populations of FAW differ between SEA countries/Australia and in terms of their population genetic diversity and current pesticide resistance profiles?

This project is an opportunity to conduct fundamental research on a pest that has become established with outbreaks across SEA and Australia, and which has increasingly been detected also in the Pacific Island and Territory Communities, and in New Zealand. The knowledge generated will be aimed at the production systems being used by smallholder farmers in SEA, to generate management options that may assist them. This project will also aim to integrate the research with activities currently being planned in Australia, aimed at Australian grain producers. This is a co-funded project with the Grains Research and Development Corporation (GRDC), with financial contributions from the Cotton Research and Development Corporation (CRDC), FMC Australia and Corteva. The activities proposed have potential to generate new knowledge that will ultimately benefit farmers in both regions.

4 Objectives

The objective of this project was to characterise FAW populations in SEA and northern Australia crop production systems as the first step towards developing long-term management options. The project therefore aimed at foundational research activities necessary for potentially scoping future research steps, focusing on: (i) understanding current FAW management options that have been shown to be promising in farming systems elsewhere such as in East Africa, and which could be applied to SEA, and (ii) characterising the FAW populations in Australia and SEA. This characterisation aims to inform and identify the likely presence of genetically unique populations, including whether there were any existing levels of insecticide resistance in the current populations, as well as the possibility of identifying biosecurity weaknesses to enable better preparedness that will be needed for on-going and future accidental introductions of exotic plant pests and diseases. Importantly, the knowledge generated will be useful for the development of future Integrated Pest Management (IPM) approaches and a draft resistance management plan. The research questions to be addressed include:

1. **What FAW management options exist or can be easily implemented in Southeast Asia?**
2. **Which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management?**
3. **Do the populations of FAW differ between SEA countries/northern Australia and in terms of their population genetic diversity and current pesticide resistance profiles?**

5 Methodology

A series of activities were co-developed with the GRDC and with SEA partners.

Activity 1: Identify and connect with partners to discuss their research priorities and terms of project engagement, and established and implemented a project communication plan for the life of the project.

Partners identified and confirmed were:

a. Uganda (Dr Andrew Kalyebi, Private Consultant, Activity 3; Dr Michael Otim, NaCRRI, Activity 2)
b. Vietnam (Plant Protection Institute of Vietnam; Activities 4, 5, 6, 7)
c. Myanmar (Department of Agricultural Research; Activities 4, 6, 7)
d. Malaysia (CABI and Malaysian Agricultural Research and Development Institute; Activities 4, 6, 7)
e. Lao PDR (Plant Protection Center; Activities 4, 6, 7)
f. Indonesia (Department of Crop Protection, University of Gadjah Mada; Activities 4, 5, 6, 7)
g. Philippines (Biology Department, De La Salle University; Activities 4, 5, 6, 7)
h. Cambodia (Cambodian Agricultural Research and Development Institute; 4, 6, 7)

Objective 1: What FAW management options exist or can be easily implemented in Southeast Asia?
To address this, we undertook literature review involving published work from 'grey literature' as well as peer-reviewed journal articles that addressed management options, and considered their adaptability to the SEA context. Furthermore, farmers in Africa were interviewed by project team members (AK, MOH) based in Uganda to ground-truth the practices presented in the literature. FAW management practices from partner countries in SEA countries were also identified by project partners for their local regions and catalogued to enable overall evaluation against practices in Africa and SEA.

Objective 2: Which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management?
The project team aimed to conduct a basic survey of crop regions that differ in their production practices, and to monitor for FAW on a variety of crops/host plants at multiple points in time (not using pheromone traps, checking plants), with FAW samples collected to be used for addressing research question 3. We anticipate that not all countries will conduct a survey, and will depend greatly on the ability to travel.

Activity 2: Desktop-based literature review on management options being studied and considered in Africa and South Asia. This will contribute to understanding how useful they may be if implemented in the SEA farming contexts. While Australian production systems are quite different there are still potential learnings for domestic FAW management. CSIRO will lead this component with input from Uganda (Dr Andrew Kalyebi, Dr Michael Otim), and where possible also input from project partners from SEA countries.

Activity 3: Documentation of practices that have been useful in Africa/South Asia for managing the pest by farmers. This may involve phone interviews/discussions with regional extension staff and champion farmers and gathering knowledge that is not included in the scientific literature (not captured in 2). This required a short contract with a scientist based in Africa to progress the work and Dr Andrew Kalyebi (Uganda) was contracted to undertake this component.

Objective 3: Do the populations of FAW differ between SEA countries/northern Australia and in terms of their population genetic diversity and current pesticide resistance profiles?
This work would build on the concurrent molecular characterization work planned under the GRDC partnership. There are three components, involving:

(i) A genomic approach following the methodologies detailed in Guan et al. (2021), Tay et al. (2021a; 2021b) and Tay et al. (2022b) and literature review to survey mutations associated with known insecticide/Bt resistance genes. Whole genome sequencing will utilise specimens from SEA and Australia, as well as from East Asia (i.e., South Korea).

Reported resistance allele frequencies relating to ACE-1 (also referred to as AChE) in the invasive FAW populations were reviewed to gain a global overview of potential spread patterns in Africa, Asia and Oceania. This work has been reported in Tay et al. (2021a) and new results from Australia, PNG, and South Korea being prepared for publication (Tay et al. 2022b). Whole genome sequencing (WGS) approach on FAW individuals were used to generate FAW genome resources that were mined for strain ID and resistance genes using known mitochondrial (mtDNA) markers and resistance alleles identified in the literature. Allele frequencies summaries from whole genome sequencing and literature review were summarised and presented in Fig. 1.

We further developed a metagenomics approach based on the Illumina MiSeq high throughput sequencing platform as proof-of-concept to determine the proportions of C-strain vs. R-strain following the methods described in Edwards et al. (2018) and Tay et al. (2022c) and to concurrently estimate the resistance gene allele frequencies for the VGSC gene and the frequencies of C-strain and R-strain individuals in Vietnam and Australia FAW populations. Primers for the metagenomics approach were designed using the primer designing software Oligo 7. Primers for FAW strain identification was based on the partial mt*COI* gene (SfHTScox1-F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATGGAACTCAAATYAATTATTC; SfHTScox1-R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCGTTGATTAATTATAGATTCTC), and on the relevant ACE-1 gene region where point mutations underpinning resistance to organophosphate insecticides were known (SfHTSace1-F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGATCAGCTGATGGCTTTACAATGGG; SfHTSace1-R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGTTCTGATGTGCTAATGATCGAG). ACE-1 allele frequencies and FAW strain identity from Vietnam and Australia were summarised and presented in Fig. 2.

(ii) Insecticide and Bt bioassay approaches to be conducted for FAW populations from various SEA countries and will involve two FAW populations from two separate regions of Australia (Western Australia (WA; CSIRO colony Sf20-4), Queensland (Qld; CSIRO colony Sf20-1)) using predefined protocols to ensure results are comparable between SEA and Australia. Detailed bioassay protocols have been provided in Tay et al. (2021a) and Tay et al. (2022b).

Partners chose to run a unified protocol in their own country, and the development need of specific protocols were identified and communicated to the CSIRO team who provided technical backstopping and guidance. Note that the GRDC also seek co-investment from Australian partners for certain aspects of this work. Insecticides of specific interest to the SEA partners were:

a. **Indonesia:** Able to do bioassays of all six insecticides and if provided, also Cry1Ac, Cry1F, Cry2Ab, VIP3A.
b. **Malaysia:** in collaboration with CABI/MARDI, to carry out Emamectin Benzoate, Alpha-Cypermethrin, Chlorantraniliprole, and Indoxacarb bioassays (Bt was proposed as optional and was not carried out).
c. **Philippines:** interested to do bioassays for two *Bacillus* strains they have locally (*B. subtilis*, *B. amyloliquefaciens*), and also chlorantraniliprole (Table 6).
d. **Vietnam:** Interested to test Cry1Ac, Cry1F, Cry2Ab, and VIP3A, and Emamectin Benzoate, Indoxacarb, Spinetoram (but these were not carried out).
e. **Lao PDR:** expressed interest to do bioassays and tested two insecticides (alpha cypermethrin and indoxacarb).
f. **Cambodia:** Interested to test insecticides widely available, including Cypermethrin, Chlorfenapyr, Emamectin benzoate, and Indoxabarb, as well as expressing an interest to test out some Bt’s. However, due to significant impact from the COVID-19 pandemic, as well as significant adverse climate events a separate report was prepared (see Appendix 2) as a substitute for the planned bioassay activities.
g. **Myanmar:** of the insecticides selected for bioassay experiments in Australia, only limited chemicals are registered. Myanmar would be able to do Emamectin Benzoate, and requested to send other insecticides from Australia for testing. Myanmar also indicated an option would be to test efficacies of entomopathogenic fungi *Metarhizium*. No up-date from the Myanmar partner following the Australian Government’s sanctions on the Myanmar military regime.

(iii) Infer population structure and diversity via analyses of genome-wide sequence data and will follow the methodologies provided in Tay et al. (2022a), Tay et al. (2021b), and Rane et al. (2022a).

We will use standard protocols for genomic DNA extraction, WGS DNA library preparation, and commercial genome sequencing service providers to generate the genome data needed for the proposed genome analyses. Genome analyses used commercial bioinformatic genomic analysis programs and were based on analysis and interpretation of relevant published scientific literature, especially relating to resistance genes.

Activity 4: A comparison of FAW populations in year-round production areas versus areas with defined production windows using a survey approach. This could be focussed on one country in SEA with a comparison to a region in Australia. Samples collected could be used as part of the molecular characterisation in 5 and may include a range of potential host plants.

Details: Dr Nguyen and Dr Hang (PPRI, Vietnam) have agreed to participate in a comparison of FAW populations based on agro-climatic settings (e.g., between Northwest, Red River Delta, North Central Coast, South Central Coast, Central highlands, Southeast, Mekong Delta). In the first instance PPRI suggests Northwest and Central highland as survey sites and which will consider factors such as crop culture practices, rotation and alternative hosts. The Australian comparisons will be limited to the northern Australian regions where FAW populations have successfully established and where suitable crop hosts will be available.

Activity 5: Develop a molecular characterisation protocol to test samples of FAW from partner countries, samples entering Australia, and samples from north Australia. This may require genomic differentiation which could be provided by a commercial sequencing company. It may be that some partners would like to develop their capacity to run the bioinformatics pipeline, so they can interrogate their samples in the future. This includes assessing samples for known resistance alleles expanded to include SEA partners.

Details: Molecular characterisation of FAW resistance genes from SEA and Australia populations will be characterised following the methods of Guan et al. (2021) via whole genome sequencing (WGS) data. The molecular characterisation of the FAW resistance genes from all populations provided by SEA partners have been reported in Tay et al. (2021a), while PNG, South Korea, and Australia FAW resistance gene characterisation and bioassays of Australia lab colonies maintained at CSIRO BM were also reported (Tay et al. 2022b).

For population genomic analyses of SEA, East Asia (EA) and Australia FAW populations, we followed the methods described in Tay et al. (2022a), involving the use of a well-characterised set of reduced genome representation of single nucleotide polymorphic (SNP) markers to map invasive FAW populations to native New World FAW populations from the North, Central and Southern Americas, and to infer gene flow directionality and identify aspects of invasion biology such as frequencies of founder events, signatures of independent vs. natural introductions, and factors that underlined FAW invasive populations' spread patterns when compared with African and Asian FAW populations of Tay et al. (2022a). We also determined population connectivity between FAW populations from SEA and Australia, and inferred likely pathways and frequencies of introductions (Rane et al 2022a). We annotated the full mitochondrial DNA genomes of the target FAW populations to infer strains (i.e., C-strain, R-strain) and estimated the minimum number of maternal lineages (Rane et al 2022a).

Activity 6: Bioassays on different populations of FAW against a panel of likely insecticide options to test for resistance expanded to include SEA partners. This should include Bt toxins as well. It may be that partners would like to run a unified protocol in their own country and this would need to be developed and communicated. The CSIRO team would provide technical backstopping and guidance. Ideally, the findings of the bioassays should be comparable across different research teams via the use of a rigorous and well-defined protocol. Note, GRDC is also seeking co-investment from Australian partners for certain aspects of this work.

Details: While all SEA partners have agreed to provide FAW samples for whole genome sequencing purpose to enable population genomic analysis and molecular characterisation of resistance genes, difficulties with respect to sharing of material especially between Cambodia/Australia and Indonesia/Australia were encountered due to local government's policies relating to exporting biological material. The impact of the pandemic has resulted in delays in shipment of FAW from various SEA partners, while inconsistent local practices by courier companies (even by the same companies located in different countries) relating to transportation of flammable liquids, i.e., the high (≥95-99.9%) ethanol used to preserve the FAW specimens, have presented also significant challenge to achieving the project aims.

The FAW samples representing first interceptions in Australia from the Torres Strait Saibai and Erub Islands, as well as first mainland Australia FAW populations from northern Queensland were requested from QDAF and DAWE and brought to CSIRO BM site. The conditions of samples being preserved, and the very limited quantities of material shared by DAWE and NAQS nevertheless prevented utilisation of these highly informative early-intercepted specimens, especially from Saibai and Erub Islands, and from Bamaga on mainland Australia. WA and NT FAW samples were sourced, received, and analysed as detailed in Rane et al. (2022a Table S1).

SEA, EA, and Australia FAW samples were sequenced through commercial sequencing providers. Processed SNPs and full mitogenome data have been made publicly available (Rane et al. 2022a, 2022b, 2022c), and sequence data are available to all partners upon request. To-date, raw sequence data was shared with the EA (South Korean) partner through direct request to the project leader.

Activity 7: Communicate the research findings to SEA partners via involvement in regional forums and research networks. Provide recommendations to the ASEAN FAW Action Plan about future research needs. Communicate the findings via the project partners to stakeholders in SEA. This may include a final face-to-face project meeting dependent on travel restrictions in early 2021. Summaries of the meeting held in Singapore are provided (see Appendix 5).

Details

(i) CSIRO and Universitas Gadjah Mada (UGM, Indonesia) coordinated to complete all bioassay data analyses from Australia and SEA partner countries. A final face-to-face project partner meeting was held in Singapore on 23-July 2022, and included ACIAR, GRDC, ASEAN FAW Action Plan Secretariate, and CSIRO Global representatives.

(ii) CSIRO provided clear outline of planned monthly meetings over the course of the project life. Progress on research outputs was provided during the planned monthly meetings. Individual phone and web-based meetings were also held with specific partners as needed, including meetings with PPRI on field surveys, with DLSU on choice of Bt toxins for bioassays, with CARDI on alternative reports in leu of bioassays, with CABI on bioassay approaches, and with Myanmar on trouble shooting bioassays and laboratory colony maintenance, and with Uganda on design of field surveys. Monthly meeting minutes were provided to all project partners including to the ACIAR project manager.

(iii) With the lifting of travel restriction, a final face-to-face meeting (initially planned for April/May 2021) was planned in Singapore (23rd July, 2022) for its well-managed public health and safety policy relating to post COVID-19 pandemic international travels, and due to its overall central location to all partners from SEA, Uganda, and Australia. Furthermore, there is also local support from CSIRO Business Development and Global (BD&G) team based in Singapore, and ease of participation by the ASEAN FAW Action Plan Secretariat (Dr Alison Watson) based also in Singapore. The CSIRO BD&G team and Dr Watson's involvement was particularly important as they would present to ACIAR and SEA partners the DFAT funded Indo-Pacific Biosecurity RD&E Partnership Program involving the proposed setting up of an ASEAN Bioprotection Research Centre (ABRC) to be led by CSIRO.

All but one project participants were invited to attend (taking into consideration the Australian Government's imposed autonomous sanctions on the Myanmar Government) the final project meeting to communicate their findings with interest groups and with the ACIAR and GRDC funders. Other interested parties such as FAO representative, DFAT, and DAWE, were also invited to attend however were unable to make the trip. The meeting is anticipated to provide active discussion on identifying future research collaboration opportunities and influence FAW management strategies in SEA and Australia, based on our genomic and bioassay findings.

The final face-to-face Project Meeting took place on 23-July, 2022, in Singapore and a summary of the project meeting from all partner presentations is provided in Appendix 5.

6 Achievements against activities and outputs/milestones

Objective 1: To understand what FAW management options existed or could be easily implemented in SEA

Table 1:

no.	activity	outputs/ milestones	completion date	comments
1.1	Literature review on published work addressing management options and their adaptability to the SEA	• What FAW management options exist or can be easily implemented in Southeast Asia? • Which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management?	31-March, 2022	Published work reviewed and assessed; grey literature searched and reported for some. Translation from local language in some cases have been the main challenge, while integrity of reported scenarios/results have been difficult to assessed.
1.2	Contacting people who work with farmers in Africa and or South Asia to ground truth the practices discussed in the scientific literature	Uganda: • Dr Andrew Kalyebi, Private Consultant • Dr Michael Otim, NaCRRI	30-June, 2021	1. African scientists from Uganda contacted 2. ASEAN FAW Action Plan has implemented a work package to understand farmers' practices in SEA (work in progress) 3. Project partners (Malaysia, Philippines, Indonesia, Cambodia, Vietnam, Laos) interacted with local farmers and provided summary and helped with translation where needed, and helped to locate relevant grey literature.

Objective 2: To understand which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management

Table 2:

no.	activity	outputs/ milestones	completion date	comments
2.1	Conduct basic survey of crop regions that differed in production practices	ACIAR project story	31-March, 2022	• This work has been significantly impacted by movement restrictions in placed over the project time that coincided with the COVID-19 pandemic. FAW was initially surveyed only from maize as the starting point prior to travel restrictions. • Multi-timepoint surveys of FAW would require significant financial support, and is also currently undertaken by the ASEAN FAW work plan. With the easing of travel restrictions post COVID-19 pandemic, this activity should be re-visited, or should be teamed up with the work undertaken by the ASEAN FAW Work Plan secretariate (Dr Alison Watson).

2.2	Collection of FAW for genomic analysis	• BIR ms (revised and resubmitted): FAW in PNG • JEE ms. on bioassays (Tay et al. 2022b accepted) • Scientific Reports ms. (Rane et al. 2022a accepted) • GRDC final reports on: (i) bioassays of FAW (Tay et al. 2021a) and (ii) FAW population genomics (Tay et al. 2021b) • ACIAR Annual report 2021	30 June 2021 31 August 2022 31 March 2022 30 June 2021 Tay and Walsh 2021	• All partners participated and successfully collected material however not all material was successfully shared due to factors such as: (i) local government policies relating to exporting of biological material (e.g., Cambodia), and (ii) local courier companies' HS&E policies on transporting flammable liquids (Indonesia, Cambodia). • Other un-official project partners including PNG and South Korea also provided FAW material to contribute to genomic analysis of FAW from East Asia/SEA and Pacific/Oceania regions.

Objective 3: To understand if FAW populations differed between SEA countries and Australia via investigation of genetic diversity and understanding of current pesticide resistance profiles

Table 3:

no.	activity	outputs/ milestones	completion date	comments
3.1	A genomic approach to survey mutations associated with known insecticide/Bt resistance genes	• Journal of Economic Entomology manuscript accepted (Tay et al. 2022b) • ASEAN FAW Action Plan webinars (see section 8.4)	30 June 2022	• Genomic survey of known mutations associated with insecticide resistance were successfully carried out for all partner countries except for Indonesia and Cambodia due to difficulties of accessing these populations. • SEA FAW population genome patterns relating to insecticide resistances were compared with multiple Australian populations.
3.2	Insecticide bioassay approaches to be conducted using predefined protocols	• A Journal of Economic Entomology manuscript accepted for bioassay outcomes of Australia FAW populations (Tay et al. 2022b). • Bioassay results from partner countries except Myanmar and Cambodia	30 June 2022	• CSIRO provided support with respect to sharing of predefined bioassay protocols to all partners that were able to undertake their respective bioassay experiments. • Political and COOVID-19 related challenges prevented some countries to complete the insecticide bioassay experiments. For Cambodia, a report highlighting the local FAW scenarios and management effort was instead prepared (Sathya et al. 2022 in Appendix 2). • The component enabled many SEA partners that previously had not have any insecticide bioassay experience to have an opportunity to increase their research skills that will prepare them for future FAW management tasks, including networking to SEA researchers as well as CSIRO to obtain assistance should the need arises.

3.3	Infer population structure and diversity via analyses of genome-wide sequence data	• Scientific Reports manuscript prepared (Rane et al. 2022a, accepted).	30 June 2022	• Population genomic study of SEA and Australia FAW population undertaken and completed with results highlighted the overall biosecurity weakness in SEA and Asia. Evidence of multiple introductions in SEA and Asia were also identified. • a manuscript reporting the population genomics of FAW from Australia and SEA has been submitted for review (Rane et al. 2022a) • An Annual Review of Entomology manuscript was prepared and extensively utilised the project population genomics and bioassay findings of SEA and Australian populations. • Populations from Cambodia and Indonesia were not analysed. Populations from Thailand were also not obtained despite repeated requests (courier services not able to ship flammable liquid).

7 Key results and discussion

7.1 Objective 1: To understand what FAW management options existed or could be easily implemented in SEA

Literature review on published work that addressed management options and their adaptability to the SEA context showed that a range of biological control agents have been identified and/or isolated, and the range of management options considered and applied research activities carried out in various invasive FAW populations from India, Kenya, Uganda, Pakistan, China, Thailand, Australia, Indonesia.

(a) Biological control agents
Viruses (reviewed by Hussain et al. 2021; Firake and Behere 2020a, 2020b; Lei et al. 2020), entomopathogenic fungi (EPF) (Firake and Behere 2020a, 2020b; Apirajkamol et al. 2022; Rajula et al. 2021; Idrees et al. 2021, Afandhi et al. 2022, Ullah et al. 2022, Akutse et al. 2020), parasitic nematodes (Firake and Behere 2020a), and beneficial insects including spiders, earwigs (e.g., Firake and Behere 2020a, 2020b; Soysouvanh and Phathanivog 2021), stink bug, assassin bugs (*Rhynocoris* sp., *Sycanus collaris*), ants, lady beetles (Soysouvanh and Phathanivog 2021), and parasitoids (e.g., Otim et al. 2021; Soysouvanh and Phathanivog 2021).

Options to utilise parasitoids as strategies to manage FAW has only commenced in most of the SEA region in recent times, given the relatively recent realisation of the presence of the FAW in various countries. Reports of detection of hymenopteran parasitoids from FAW larvae collected from fields have emerged (e.g., Indonesia, Wahyuningsih et al. 2022; Malaysia, Mazidah Binti Mat, MADI, Pers. comm. July 2022; Laos, Soysouvanh and Phanthanivong 2021). In Laos, the use of parasitoids was trialled but found to be uneconomical and would likely be unsuitable for local farmers (Soysouvanh and Phanthanivong 2021).

Viral infections were reported in field populations of FAW from Myanmar (Khin TN, pers. comm.) and isolated from Indian (Firake and Behere 2020a, 2020b) however the lack of resource and technical support have prevented further identification of the virus identity. EPF that infected FAW larvae were also detected from Laos (Soysouvanh and Phathanivog 2021), Thailand (Rajula et al. 2021), Philippines (Navasero et al. 2019), although fungal pathogens from Malaysia targeting FAW have not been reported, and in Cambodia trials on using *Beauveria bassiana* to infect FAW larvae were unsuccessful (Sathya et al. 2022 in Appendix 2). At CSIRO, EPF and lepidopteran virus isolates are available for testing of infection efficacies in FAW and would allow whole genome characterization of candidate isolates. The work on EPF (Apirajkamol et al. 2022) and on viral and bacterial (Bt isolates) are currently underway, with the viral/bacterial work being funded by DAFF (previously DAWE).

The use of *Bacillus* bacteria including *B. thuringiensis* (Bt), *B. subtilis*, *B. amyloquefaciens* have also been trialled both in the fields and under laboratory conditions. Use of Bt was recommended as part of the IPM solution for early management of FAW in maize fields in e.g., Malaysia (Dr Mazida Binti Mat, MARDI, pers. comm.), Vietnam (see "**7. Key results and discussion:** Current FAW management options being explored and trialled in Southeast Asia: 1. Vietnam" section), and Laos, although with limited success in Laos as reported by (Soysouvanh and Phathanivog 2021).

(b) Botanical extracts
Use of botanical extracts included Neem Oil, *Tinospora cordifolia* (commonly known as 'Guduchi') extract, tobacco, chili pepper, *Aloe vera*, and *Lantana camara*. Application of these botanical extracts is sometimes augmented by other cultural practices, such as in combination with ash as practiced by farmers in Uganda. In Laos, use of botanical extracts also was accompanied by simultaneous release of beneficial/predatorial insects such as stink bug, and killing of FAW caterpillars by hand. Application of Neem in Vietnam was in conjunction with food spray (e.g., rice flour) that act to attract beneficial insects (see "**7. Key results and discussion:** Current FAW management options being explored and trialled in Southeast Asia: 1. Vietnam" section). In Cambodia, CARDI assessed the use of Neem Oil to manage the FAW however this was found to be ineffective (Sathya et al. 2022 in Appendix 2).

(c) Push-pull technique
In Uganda, farmers have reported high rates of success using the push-pull technique to reduce FAW larval damage on maize crop (see Kalyebi 2021 in Appendix 1; Kalyebi et al. 2022), while the Malaysian Department of Agriculture also provided advice to growers on the push-pull technique (see "**7. Key results and discussion:** Current FAW management options being explored and trialled in Southeast Asia: 6. Malaysia" section). There is limited information on the success rates of this technique in SEA at present, although a review of resources

available on the web showed that most of the relevant government authorities from SEA countries have provided some information on the push-pull technique (see Appendix 4).

(d) Mechanical options
These included destruction of egg masses, killing of larvae, and pupae through mechanical means, such as physically squashing/killing of eggs and larvae. Tillage of approximately 10 cm has been recommended by CABI to Myanmar maize growers as a mean to reduce survivorship of pupae, and is also practiced in Vietnam (PPRI pers comm.), although in Uganda this was not recommended (see "**7. Key results and discussion:** Current FAW control options in East Africa (Uganda, Tanzania, Kenya): Dr Michael Otim (NaCRRI, NARO) section), highlighting the inconsistency of advice provided to growers in different countries. In Vietnam, flooding of crop fields of 2-3 days have been used to kill pupae (Nguyen VL (PPRI), pers. comm.).

(e) Crop rotation, use of trap crops, and intercropping
Intercropping of maize fields with other food crops such as cassava, ground beans, pumpkin, was reported as a cultural practice in Laos (see see "**7. Key results and discussion:** Current FAW management options being explored and trialled in Southeast Asia: 3. Laos" section), and is also a cultural practice used in east Africa nations such as Uganda (see "**7. Key results and discussion:** Current FAW control options in East Africa (Uganda, Tanzania, Kenya): Dr Andrew Kalyebi (Private Consultant) and Dr Michael Otim (NaCRRI, NARO) sections) where other food legumes, soybean, groundnuts, have been planted. Crop rotation as a cultural practice in Uganda included planting with soybeans, groundnuts, potatoes, cassava, and sorghum. In Myanmar, crop rotation included growing of rice, vegetable, with maize, while in Laos crop rotation involved growing of rice (see Appendix 4).

7.1.1 Current FAW control options in East Africa (Uganda, Tanzania, Kenya):

Dr Andrew Kalyebi (Private Consultant): In Uganda, besides chemical control of FAW, other methods predominantly (1) cultural methods, (2) the use of biological extracts, and (3) Crop diversification methods, have been promising for FAW control.

1. Cultural methods include: (i) timely and adequate land preparation, often involving a primary cultivation followed by secondary cultivation after 2-3 weeks interval before planting, (ii) garden sanitation (i.e., keeping garden devoid of refuse), (iii) crop rotation, (iv) intercropping, (v) hand picking, (vi) early planting, (vii) use of organic manure as fertiliser, and (viii) a habitat management practice commonly known as push-pull strategy. The push-pull strategy involves intercropping maize (or another cereal crop) with a legume crop (e.g., *Desmodium*) and this is simultaneously intercropped with Napier grass at the periphery (edges) of the garden. The *Desmodium* intercrop acts to repel (i.e., 'push') pests away from the maize, while the Napier grass at the edges of the garden 'pulls' the pests away from the maize.

2. The use of biological (plant) extracts predominantly from *Aloe vera*, tobacco (*Nicotiana tabacum*), chili pepper (*Capsicum* spp.), *Lantana camara* and the Neem tree (*Azadirachta indica*) used alternately or in combination with ash (as catalyst) have shown promise in controlling the FAW albeit limited only to small-sized gardens due to difficulties in ascertaining the right quantities to use.

3. Crop diversification techniques have also been important allowing farmers to use biological cycles to minimize inputs, conserve the resource base, maximise yields, and reduce the risk due to ecological and environmental factors. While crop diversification practice may include multiple cropping as opposed to monoculture, mixed cropping, use of different varieties of the crop (maize), all of which are practiced with the goal of improving productivity, sustainability and supply of the ecological system. Crop diversification practice also overlaps that of cultural practices such as crop rotation and intercropping (e.g., the push pull system, both as a habitat management strategy and also for control of pests).

Dr Michael Otim (NaCRRI, NARO): Since the introduction of *Spodoptera frugiperda* in the East African region, farmers have used different methods of control FAW to mitigate the substantial losses that particularly affected maize crop in Eastern Africa. The main methods include: mechanical control, cultural practices, intercropping, and use of different crop combinations, biopesticides and insecticides.

Among the mechanical control methods recommended for and used by smallholder farmers in Africa is squashing egg masses and larvae. The cultural methods include removal of crop residues, no-tillage, applying sand, ash, or soil in the maize whorl. Regular weeding is also recommended to control FAW damage.

The component crops known to reduce FAW infestation and damage are common bean, and groundnuts. Push-Pull technology is another intercropping system in which maize is intercropped with *Desmodium*, is also being promoted in East Africa. It uses stimulo-deterrent diversionary tactic to repel gravid moths of cereal stemborers and FAW from maize due to the intercropped *Desmodium* (push), while attracting them to the trap companion plants such as *Brachiaria* and Napier grass (pull) planted around the maize plots.

Regarding biological control, countries have documented the occurrence of parasitoids, predators, entomopathogenic bacteria, viruses, fungi and nematodes. Amongst the biological control agents, ICIPE identified two *Metarhizium anisopliae* strains (icipe 7 and 68), which have been registered in Kenya for FAW control. In Tanzania, attempts have equally been made to integrate *M. anisopliae* and *B. bassiana* into diverse cropping systems for FAW management. The process of testing and registration of effective biopesticides is ongoing in other East African countries, including Uganda.

Although host plant resistance is dependable and cheap for control of FAW, and a few tolerant lines have been identified, but they have not yet been released to farmers in the region.

Chemical control is by far the most widely used method for controlling FAW. Several insecticides have been registered and recommended for control of FAW in different African countries. These include Carbamates, Organophosphates, Ryanodine Receptor modulators, Avermectins, Spinosyns, Oxadiazines, Nereistoxin and Pyrethroids. Pyrethroids and Organophosphates are the most used, followed by Avermectins, perhaps because of their availability and lower prices.

7.1.2 Current FAW management options being explored and trialled in Southeast Asia

1. Vietnam

Surveys on current management options of FAW in Vietnam was planned and initiated by PPRI Vietnam but the activity was impacted by the worsening COVID-19 pandemic situation in Vietnam, and significantly limited this activity and prevented the continuation of the field surveys. Current management options of FAW in Vietnam include crop rotations, changes in crop cultural practices, and impact from alternative hosts on reducing FAW damage are beginning to be experimented. At the sites where FAW were collected for genomic studies (see Fig. 2 of Tay et al. 2021b), cultural practices included crop rotations of rice, beans, and vegetables, all of which are known alternative host plants for FAW. Cultural crop practices have also undergone changes, with increase in plantation of maize capable of expressing *Bacillus thuringiensis* (Bt) Cry toxins (i.e., Bt maize).

2. Indonesia

In Indonesia, it is very rare to see intercropping corn with other crops. However, corn may be planted in different agroecosystems. The following are results from the Trisyono group (Universitas Gadjah Mada) from a study assessing the role of egg parasitoids on mortality of FAW eggs in three different corn ecosystems: corn within agroforestry; corn in the irrigated rice fields, and corn in the rainfed fields. Two major egg parasitoids were found: *Telenomus* sp. and *Trichogramma* sp., with *Telenomus* sp. being found more abundantly in the three ecosystems, although no molecular species diagnostics have been carried out to further confirm species status of the parasitoids. The egg mass parasitisation varied from 15.6 to 52.5%, and the number of egg masses parasitized was consistently higher in agroforestry, followed by rice fields and rainfed fields. These results may provide the basis for ecological engineering to increase the ecosystem services (Whayuningsih et al. 2022).

3. Laos

There is limited resource and information relating to IPM methods for the management of FAW in Laos. According to the report prepared by Soysouvanh and Phanthanivong (2021), FAO, the Lao farmer Network, and the Lao Upland Rural Advisory Service (LURAS) have supported various farmer education projects on IPM and on understanding the biology of this exotic insect pest. FAO supported the Training of Trainers (ToT) training program that included resource posters on diagnostics of FAW including larval and adult moth morphological characters and damaged crop symptoms caused by larvae, and IPM options using natural enemies and biocontrol agents for the management of FAW. The Lao farmer Network and the Lao Upland Rural Advisory Service (LURUS) undertook research on FAW management in farmers' fields, although no results have been made public at the time of preparing this report. Other ToT resources have also been prepared and shared as PowerPoint presentations and included IPM of FAW management, resistance maize varieties, using pheromone and molasses to trap and control FAW, natural enemies and raring of natural enemies, and production and using bioagents for FAW management.

Farmers manage FAW in maize planted as animal feed have used insecticides to control the FAW. On farms that is considered as big (≥ 1 ha), insecticides were applied on crops up to 1-2 months old, and farmers (especially those involved in LURUS project from the Xiengkhoung Province) also attempted to use beneficial insects such as stink bug to control FAW. For small farms where farmers planted maize for human consumption and to be sold in local villages and communities, both insecticides and crop rotation with rice and vegetables were practiced, especially in areas around Vientiane capital and Vientiane Province. In the south of Laos (Salavan Province), small scale farmers that grew maize for human consumption and that are being sold in the village reported that they used insecticides to manage the FAW, while also integrated crop with ground bean, pumpkin, and cassava. The efficacies of these intercropping and crop rotation practices to manage the FAW is not known.

4. Philippines

Through the Memorandum Order 26 issued by the Department of Agriculture (DA) on March 31, 2021. The Philippine government launched the Fall Armyworm (FAW) Program as a campaign to stop the FAW outbreak. The program includes pest monitoring and intervention efforts in managing the population of FAW. To sustain the campaign against FAW, DA created the inter-agency national FAW task force. The task force is leading the implementation of strategies and measures to effectively control FAW infestation through strict quarantine inspection, disinfestation of ports, sanitation, cultural management practices, distribution and use of pesticides and biological control agents, and strategic information dissemination. The government trained and capacitated farmers to identify FAW and its damage for early detection and to facilitate removal of infested plants. Both the practice of crop rotation and field sanitation have also been encouraged by the government although their efficacies under Philippine's agroecological systems as management options against the FAW are as yet unknown. Additionally, the Department of Science and Technology-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) funded various basic and applied research projects to address the FAW outbreak in the Philippines, including (i) effect of temperature and host plants on the life history traits of FAW, (ii) genetic structure and morphological variation analyses of the FAW, (iii) Biological control of FAW using entomopathogens; (iv)identification and preliminary evaluation of natural enemies against the FAW; (v) development of an early warning system against FAW through phenology and distribution modelling, and (vi) FAW insecticide management and susceptibility studies.

5. Cambodia

In Cambodia, there is limited understanding and a lack of scientific research on the impact, pest genomics, and potential options that exist for managing the fall armyworm *S. frugiperda*. Damage level and pest incidences have been conducted via field surveys in various provinces, and attempts to explore management of this pest using Neem Oil as well as using the entomopathogenic fungus Beauveria bassiana were carried out in laboratory experiments but these were unsuccessful. A separate report on the current IPM activities undertaken by CARDI during this project period is provided in Appendix 2.

6. Malaysia

Current FAW management options outlined below has been contributed by Dr Mazidah Binti Mat and her research team (Tang Siew Bee, Saiful Zaimi Jamil, Zulaikha Mazlan, Wan Khairul Anuar Wan Ali, Norzainih Jasmin Jamin, Mohd Masri Saranum and Wan Muhammad Azrul Wan Azhar), from the Pest & Disease Management Programme, Industrial Crops Research Centre, MARDI, and with funding support by MARDI Special Research Grant (Grain corn project KRL-167/KGB-167-1001) awarded by the Ministry of Agriculture and Food Industries).

Since the confirmation of the FAW in west Malaysia (e.g., Perlis state in Feb 2019; Johor state in June 2019, Kelantan in August 2019) and east Malaysia (Sabah and Sarawak, December 2019) IPM approach to manage this invasive pest was trialled. Prior to the implementation of an interim IPM approach, pest infestation and crop damage in four maize growing sites were moderate to significant (e.g., Changlun in Kedah state: 100% incidence of FAW in 2019, damage >50% (considered as severe damage); Labis in Johor state, 100% FAW incidence in 2019, damage severity level of >50%; In Sik (Kedah state), 2019 FAW incidence was 20% and with moderate level (30%) of damage; In Chuping (Perlis), incidence of FAW in 2020 was 30% and with 20% (moderate level) of damage).

MARDI conducted field visits to farms attacked by FAW between 2019-2020 to share knowledge and expertise with farmers on FAW management. MARDI also developed an interim IPM approach against the FAW in Malaysia, with the aims to: (i) conduct a baseline and scoping analysis of FAW infestations situation and levels on maize and other economically important hosts (i.e., rice) production sites, (ii) conduct laboratory and field evaluation on pheromones, biopesticides, and chemical insecticides for control of FAW, (iii) formulate and evaluate an interim IPM program in based on plant developmental stage-based approach supported by action thresholds, and (iv) to make recommendation for IPM implementation for up-scaling and sustainability for awareness and capacity development.

Proposed interim IPM for FAW management in grain corn and sweet corn included: use of pheromone traps (purchased from Costa Rica) for early monitoring of FAW (at 14 Days after planting (DAP)); at 7-14 DAP use of Bt spray when FAW incidence level was at 5-20%, use of Emamectin benzoate/Chlorantraniliprole if more than 20% incidence; 15-28 DAP 5% incidence to spray with Emamectin benzoate/Chlorantraniliprole; and at 29-49 DAP with 5% incidences to spray with Emamectin benzoate/Chlorantraniliprole. Chemical spray would involve alternating the insecticides, and when at tasselling stage and onwards there would be no insecticide spraying.

Beneficial insect populations were monitored in trial plots, and adult FAW moth populations monitored using pheromone traps, with insecticide spray according to pest incidences at different maize developmental stages. The interim IPM approach led to reduced pest populations and reduced severity of crop damage, produced higher crop yield, and increased beneficial insect population in IPM trial plots when compared with control plots. There is a need to continue to monitor for development of resistance to Emamectin benzoate and Chlorentraniliprole insecticides although their alternating use can help to reduce development of insecticide resistance in the FAW populations. There is also a need to explore the use of biological control agents to complement the interim IPM strategy.

7.2 Objective 2: Which crop/plant diversification options are available to increase resilience in maize production systems that assist with integrated FAW management?

A range of crop/plant diversification options have been trialled in Uganda and in Southeast Asia, although at least for Southeast Asia these were not necessarily specifically developed as a cultural option for the management of FAW, and could be traditionally grown. In East Africa such as in Uganda, farmer field surveys identified cassava, potato, sorghum, groundnut, and soybean as diversification options. In Southeast Asia such as in Myanmar, rice and vegetable were grown, while in Laos, cucurbits, rice, ground beans, pumpkins, and cassava were grown. While majority of countries in Asia, Africa and Pacific/Oceania have reported maize as the main crop attacked by the FAW, increasingly attack on other crops are also being reported, including sugarcane in India (Chormule et al. 2019), China (黄 2019), barley in China (Yang et al. 2019), ginger in India (e.g., Firake and Behere 2020b) and Australia, while in recent times also rice in Philippines (Anamalai S. (CABI SE Asia) pers. Comm.) and India (Kalleshwaraswamy et al. 2019). Attack on cassava by the FAW is not known in Africa and SE Asia despite the popularity of growing cassava as a major economic crop. Crop plant diversification to help with managing FAW therefore remains a research area that requires research.

7.3 Objective 3: Do the populations of FAW differ between SEA countries/northern Australia and in terms of their population genetic diversity and current pesticide resistance profiles?

This work would build on the concurrent molecular characterization work planned under the GRDC partnership. There are three components, involving: **(i)** a genomic approach to survey mutations associated with known insecticide/Bt resistance genes using specimens collected from SEA and Australia cropping producing regions; **(ii)** insecticide bioassay approaches to be conducted using predefined protocols to ensure results are comparable, and **(iii)** infer population structure and diversity via analyses of genome-wide sequence data (further methodology details provided in section **5. Methodology:** Objective 3: Activity 5)

7.3.1 Molecular characterization of known resistance genes and alleles

Molecular characterisation of the FAW genome at the population scale across various invasive populations from Australia and from project partners showed that resistance alleles to carbamate/organophosphate pesticides were widely detected. Results of molecular characterisation of insecticide resistance genes have been reported in Tay et al. (2021a; 2022b). While the scientific and agricultural communities have, to-date, tended to agree with the assumed west-to-east spread of FAW that likely started from western Africa, population genomic evidence supported instead that eastern African FAW likely originated from Southeast Asia, with multiple FAW introduction events occurring in Southeast Asia and Asia as also supported by unique allele frequencies in the ACE-1 gene (Fig. 3; see also Tay et al. 2021b). Whole genome sequence mapping of the ABCC2 gene that is a known target gene for the Bt toxins Cry1Ac and Cry1F (e.g., Banerjee et al. 2017) also identified a putative two base pair deletion in one of the gene exons that could lead to premature stop codon and the loss of function to the gene and potentially resistance to these Cry toxins (Tay et al. 2022b). The approach of WGS to further characterise mutations in the ABCC2 gene in the current global FAW populations especially in invasive populations should be a priority as countries in Southeast Asia (e.g., Vietnam, Philippines) and Asia (e.g., China) have begun to adopt more widely transgenic maize and rice as a management strategy against the FAW (Silvie 2022).

Reported candidate resistance alleles to organophosphates and carbamate pesticides in ACE-1 (acetylcholine esterase gene) have been identified in the surveyed FAW populations. Two of the three alleles (i.e., A201S, F290V) are present in both the native (i.e., the Americas; Appendix 3) and invasive (i.e., rest of the world; Appendix 3, Fig. 3) range while one is not found in the individuals from the Southeast Asian and Australian invasive populations examined. The G227A allele was only present in Indonesian and China Hubei populations based on summary of published data (Fig. 3). No known resistance alleles were identified to pyrethroid or group 28 pesticides in the invasive populations sampled across Southeast Asia and Australia in this study.

Resistance alleles to carbamate/organophosphates were identified in the populations. The most common resistance allele detected in the invasive and native population (from 456 individuals) was the F290V mutation with 66 homozygous and 222 heterozygous resistant genotypes detected. The second most common allele detected was A201S (from 456 individuals) with 90 heterozygous individuals detected. The G227A mutation was the least common (from 456 individuals) with 21 heterozygous individuals and 3 homozygous individuals detected respectively. F290V was present in all locations in both the native and invasive range suggesting that most populations will contain this mutation. A201S was present almost everywhere but at much lower frequencies including in Australia. Interestingly G227A was only present in individuals from the native range (Brazil, USA, Puerto Rico, Peru), from one Indonesian population from Kediri Java (D. Bauventura pers. comm.; Bauventura et al. 2020a), and four populations from the Hubei Province in China (Guo et al. 2020), and is likely rare in the invasive range (Tay et al. 2021a, Tay et al. 2022b).The ACE-1 resistance allele frequencies identified by our study are comparable with reported allele frequencies from across the FAW invasive range to-date, including for other Australian populations reported so far (e.g., Nyguyen et al. 2021).

No alleles predicted to cause resistance to pyrethroids or the group28 pesticides were identified in this work. However, while the previously identified resistance alleles were not identified, considerable variation was identified in the RyR gene at the potential resistance loci (data not shown). This should be further investigated in conjunction with bioassays to establish whether any of the variants could contribute to resistance.

Metabarcoding & resistance alleles surveys by Hight throughput amplicon sequencing

We have developed a metabarcoding approach to survey FAW population diversity composition and three ACE-1 alleles that conferred organophosphate resistance status in field-collected FAW populations. This approach was demonstrated using Vietnam field-collected populations vs. Australian WA populations (Fig. 1). The metabarcoding approach was based on the African field-survey of *Bemisia tabaci* cryptic species composition approach (Tay et al. 2022c). We applied the 5’ terminal mt*COI* partial gene region that exhibited greater nucleotide substitution sites than the widely used standard (i.e., 3’ terminal) barcoding region, thereby offering the opportunity to also estimate the number of maternal lineages through estimating the number of unique COI haplotypes detected if so desired. For understanding insecticide resistance status, we demonstrated the metabarcoding approach through estimating field resistance status to organophosphate via characterisation and estimating frequencies of A201S, G227A, and F209V variants in each population. Estimates of resistance alleles via the metabarcoding approach has been shown to be possible in the two-spotted mites *Halotydeus destructor* (Edwards et al. 2018). We demonstrated that an easy-to-use analysis pipeline based on a commercial genomic software could be readily applied to our metabarcoding data, while acknowledging that the analytical procedures can be further refined (e.g., to include a quality trimming step) and/or to be tested out also on other genomic software. While we have only included from each population 15 individuals, future analyses could increase the number of individuals from each field to be surveyed (e.g., >30-100 individuals), include other known resistance genes (e.g., RyR, VGSC, ABCC2), while inclusion of a universal lepidopteran mt*COI* barcode primer pairs as internal control to detected accidental inclusion of non-FAW species could further improve the robustness of this HTS approach. With evidence of multiple introductions in the Malaysian FAW populations, as well as to further investigate potential links of Myanmar FAW populations with Chinese FAW populations, additional metabarcoding work could be readily undertaken using samples already provided by our Southeast Asian partners.

Fig. 1: FAW sampling sites in: (a) Viet Nam, and (b) Western Australia for trials in characterisation of the organophosphate/carbamate resistance acetylcholinesterase (ACE-1) gene and associated allele frequencies, and compositions of corn/rice preferred FAW based on the NGS approach. Ten populations from the three main agroecological zones of Viet Nam were sampled. Viet Nam population codes are also used in Fig. 2. Viet Nam map adopted and modified from the United States Department of Agriculture (USDA) Foreign Agricultural Services and the Global Agricultural Information Network (GAIN) report VM2019-0017, showing the accumulative *Spodoptera frugiperda* infected area as of 25 July 2019 and their respective ecological climatic zones.

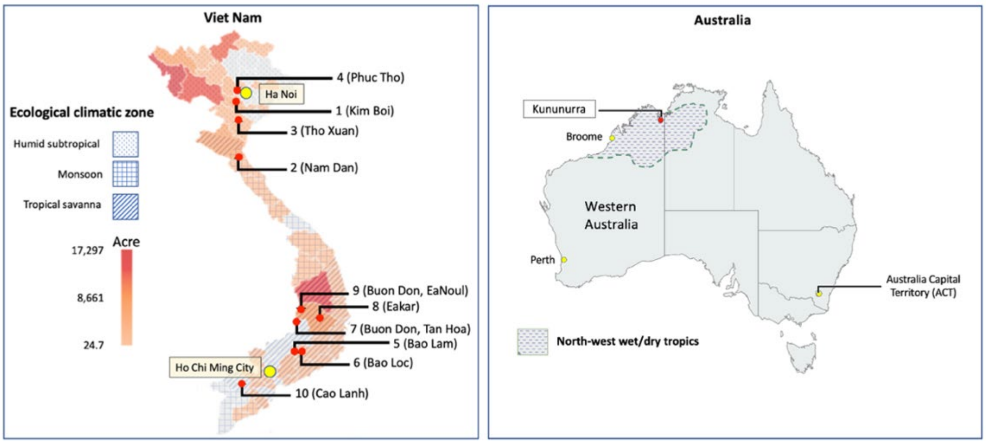
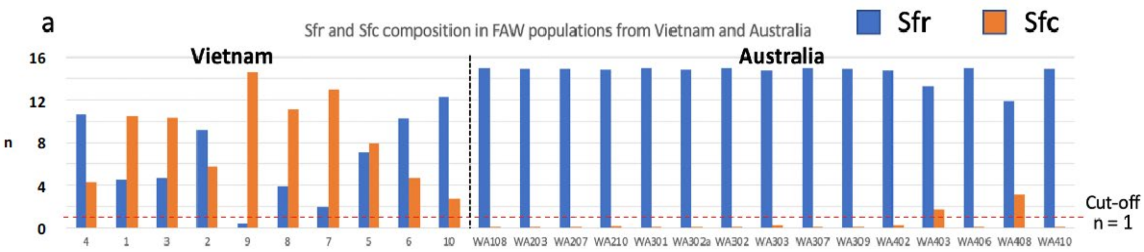
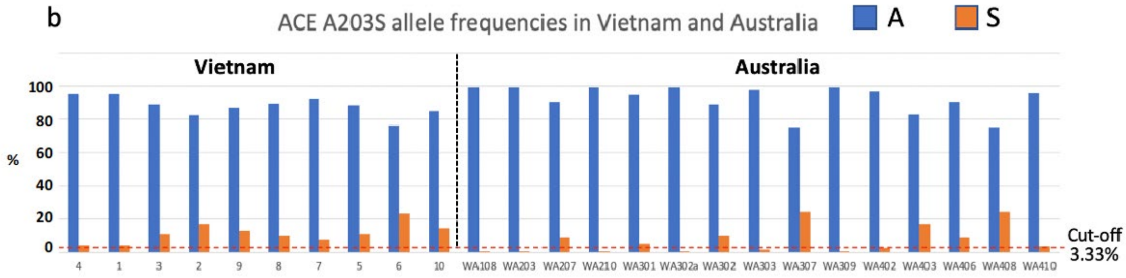


Fig. 2: Assessment of Vietnamese and Australian C- and R- strains FAW, and the ACE-1 resistance gene profiles by the NGS approach. (a) *Spodoptera frugiperda* individuals being assigned as either R-strain (Sfr) or as C-strain (Sfc) by DNA marker characterisation. Cut off for R-strain or C-strain DNA marker is set at n=1 (i.e., estimates of <1 individual is considered as false detection). (b) NGS estimates of the ACE-1 A203S resistance allele frequencies in FAW populations from Viet Nam and Australia. 'A' and 'S' are susceptible and resistance alleles, respectively. Allele frequencies of <3.3% is considered as false detection (i.e., due to potential Polymerase Chain Reaction (PCR) errors introduced during laboratory preparation of DNA), and (c) NGS estimates of the ACE-1 F209V resistance allele frequencies in FAW from Viet Nam and Australia. 'F' and 'V' are susceptible and resistance alleles, respectively. All populations tested by the NGS approach did not possess the ACE-1 resistance A allele at the G227A site (results not shown).




b
ACE A203S allele frequencies in Vietnam and Australia
A
S
Vietnam
Australia
%
100
80
60
40
20
0
4
1
3
2
9
8
7
5
6
10
WA108
WA203
WA207
WA210
WA301
WA302a
WA302
WA303
WA307
WA309
WA402
WA403
WA406
WA408
WA410
Cut-off
3.33%

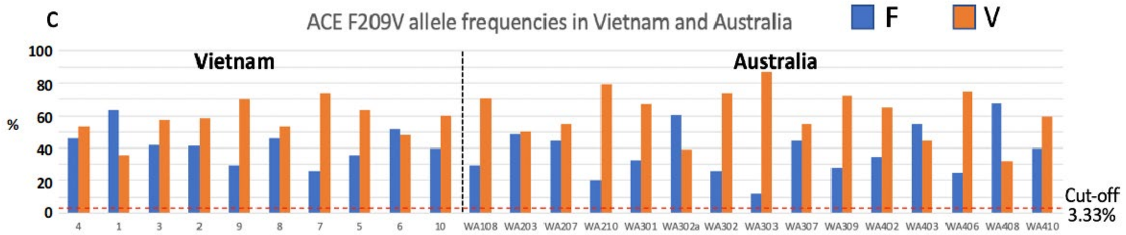
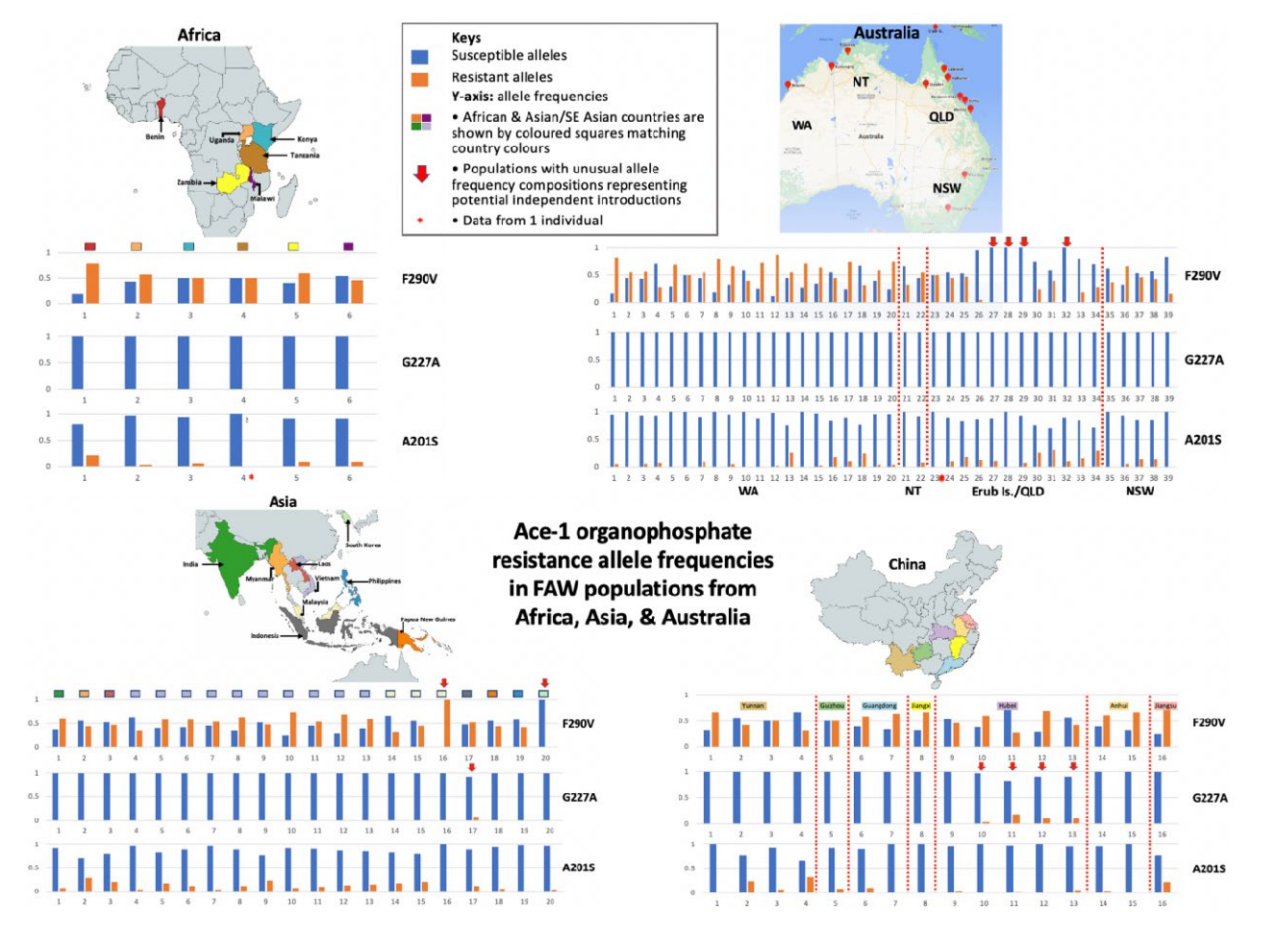

c
ACE F209V allele frequencies in Vietnam and Australia
F
V
Vietnam
Australia
%
100
80
60
40
20
0
4
1
3
2
9
8
7
5
6
10
WA108
WA203
WA207
WA210
WA301
WA302a
WA302
WA303
WA307
WA309
WA402
WA403
WA406
WA408
WA410
Cut-off
3.33%

Fig. 3: Summary of Acytylcholinesterase (ACE-1) susceptible and resistance allele frequencies in *Spodoptera frugiperda* populations from 17 countries (i.e., Benin, Uganda, Kenya, Tanzania, Zambia, Malawi, India, Indonesia, Papua New Guinea (PNG), South Korea, China, Australia) across the invasive range. A total of 1,246 individuals representing 76 populations (see Appendix 3) were used to compile the data below, and included combined data from Tay et al. (2022b) for Australia SF20-1 and SF20-4 PNG, and South Korea; and from published studies (Boaventura et al. 2020a, Guan et al. 2021, Zhang et al. 2020, Zhao et al. 2020, Nguyen et al. 2021, Tay et al. 2022b, Yainna et al. 2021, Rane et al. 2022a), as well as other SEA populations (Tay et al. 2021a).



Susceptible and resistant alleles from the three previously reported loci (i.e., F290V, G227A, A201S) from the ACE-1 gene provided evidence to support multiple independent introductions across the invasive FAW populations, such as in Indonesia (#17; G227A), South Korea (#20; F290V), and China (Hubei province (#10-13); G227A) as indicated by the red arrows. In Australia, newly established FAW populations between Queensland (e.g., #27, #28 (Walkamin), #29 (Strathmore), #32 (Burdekin), and Western Australia (i.e., #2 (Kununurra)/Northern Territory (e.g., #21 (Bluey's Farm)) suggested this likely involved multiple introductions from diverse populations from neighbouring countries and likely arrived via separate pathways and entry points. (Figure from Tay 2021a).

7.3.2 Chemical insecticide and *Bacillus thuringiensis* (Bt) toxin/protein Bioassays - Summary of Bioassay outcomes from Australia FAW populations

The two laboratory cultures of *S. frugiperda* (Sf20-1 and Sf20-4) responded appropriately to the pesticides as did *H. armigera* and *S. litura*, and we have been able to generate accurate and reliable LC_{50} data. However, for alpha cypermethrin, methomylm Chlorantraniliprole, and Indoxacarb tested (Tables 4 and 5), *S. frugiperda* requires a higher dose than *H. armigera* suggesting that: (i) either it is naturally more tolerant of these pesticides as compared to *H. armigera*, or (ii) it has arrived in Australia carrying pre-existing insecticide resistances. The difference between the two *S. frugiperda* colonies is suggestive, in particular, the scale of the difference in sensitivity to alpha-cypermethrin and indoxacarb could indicate the presence of different resistance alleles in the Sf20-4 colony.

It is possible that the variation observed is simply natural variation however it is also possible that a different incursion involving different source populations or different local selection pressure has occurred in Northern WA as compared to the eastern states. An alternative explanation, albeit unlikely, would be that different selection pressures on the global population that recently originated from the west-African incursion, have resulted in the different phenotypes being detected in our and various other published bioassay experiments. A further alternative explanation is that the differences are reflective of fitness in the different colonies based on the individual founding genetic diversity (i.e., potentially less genetic diversity in Sf20-4 due to the smaller number of F_0 individuals *cf*. Sf20-1). However, if this was the case one might expect Sf20-4 to always be less sensitive but for the Bt proteins and spinetoram and benzoate, this has not been the case.

For the development of FAW resistance management strategies, knowledge of gene flow between Australian populations from different regions will provide support to the suitability of developing state-level vs. national level resistance management strategies. On-going monitoring and bioassay testing of new populations, e.g., those from Hubei (Guo et al. 2020) or Indonesia (Boaventura et al. 2020a) that carry the ACE-1 G227A resistance allele; or the RyR (e.g., Boaventura et al. 2020b) or ABCC2 (e.g., Yainna et al. 2021; Guan et al. 2021; Flagel et al. 2018) resistance genes from the Americas arriving in Australia, is also recommended to provide the grains and related agricultural industries with preparedness to emergence and arrival of novel resistance traits.

Bioassays - Comparing between different FAW populations from SEA and Australia

Six synthetic chemical insecticides and four *Bacillus thuringiensis* (Bt) toxins were tested against larvae of *Spodoptera frugiperda* collected from the District of Klaten from Central Java, Indonesia (Tables 4, 5, 6). Larvae from one population of FAW from Vietnam were also tested against the four Bt toxins (Tables 4, 5, 6), while FAW populations from Malaysia, Philippines, and Laos were tested against selected synthetic chemical insecticides (Tables 5, 6). The Philippines FAW population was also tested against three separate Bt toxins (Table x-6). Insecticide and Bt bioassay findings of the Australian FAW Sf20-1 and Sf20-4 laboratory lines have previously been reported in Tay et al. (2021a, 2022b) but are here included to facilitate meaningful comparisons and to provide an overall picture for the responses of different FAW populations from the SEA and Oceania regions.

Bioassays were conducted using three different methods: topical application for alpha-cypermethrin and methomyl; diet incorporation for emamectin benzoate, chlorantraniliprole, indoxacarb, and spinetoram; and diet overlay for *B. thuringiensis* toxins Cry1Ac, Cry1F, Cry2Ab, and VIP3a. Because of different bioassays, toxicity of insecticides was compared among selected SEA and Australia FAW populations that were also tested using the same procedures.

In the Indonesian FAW population, Cry 1F and VIP3a exhibited similar level of toxicity, and they were significantly more toxic (*ca.* 45 times) than Cry1Ac and Cry2Ab which the toxicity of these last two were comparable. These results provide the baseline of the toxicity of the tested insecticide on the Indonesian population of *S. frugiperda* collected after the invasion to the country, and this population might not have received long field selection from insecticide applications. Interestingly, the Indonesian FAW population was the most susceptible invasive population when compared among the Vietnam and the two Australian FAW populations for the *B. thuringiensis* toxins Cry1Ac and Cry1F. Contrasting this, the Vietnam FAW was 8 times more sensitive to VIP3a than the Indonesian FAW (Table 4).

For the Indonesian FAW population studied, based on the LC_{50} values, alpha-cypermethrin was 1.7 times more toxic than methomyl against the third instars and there was no overlapping between 95% CI values of those insecticides (Table 5). Employing the diet incorporation, emamectin benzoate was the most toxic followed by spinetoram, chlorantraniliprole, and indoxacarb. Overall, the 95% CI of the LC_{50} values of these four insecticides did not overlap each other indicating that they were significantly different (Table 6). It is worth noting that indoxacarb was 368 approximately times less toxic than emamectin benzoate when tested in the same Indonesian FAW population, and approximately 626 times less toxic for the Australian Sf20-4 population from Western Australia (Table 6).

Comparison with other populations showed that the Indonesian population of *S. frugiperda* used in this study has the second highest tolerance to indoxacarb, with the Western Australia Sf20-4 line being the highest (Tay et al. 2021a; 2022b). Across the three FAW populations from SEA (i.e., Laos, Malaysia, Indonesia) tested for Indoxacarb, tolerance level was the lowest for the Laos population followed by the Malaysian population based on LC_{50} values (*ca*. 14 times and 10 times lower than the Indonesian FAW population, respectively). However, while the Loas and Malaysian FAW populations had similar Indoxacarb tolerance level as the Queensland Sf20-1 population (i.e., 1.9 times and 1.4 times, respectively), they were 17 times and 13 times less tolerance than the Sf20-4 Western Australia FAW laboratory line (Table 6).

The populations of *S. frugiperda* from Indonesia, Malaysia, and Philippines showed similar level of susceptibility to chlorantraniliprole with the LC_{50} values varied from 0.030-0.056 µg/ml diet. However, the population from Indonesia was approximately 10 times more resistant to indoxacarb (LC_{50}= 8.840 µg/ml diet) than those from Laos (0.642 µg/ml diet) and Malaysia (0.910 µg/ml diet) but the Indonesian population was five times more susceptible to emamectin benzoate (LC_{50}= 0.024 µg/ml diet) than that from Malaysia (LC_{50}= 0.110 µg/ml diet). On the other hand, the population from Laos (LD_{50}= 1.660 µg/larva) was approximately 8-10 times more resistant to alpha cypermethrin than those from Malaysia (LD_{50}= 0.2 µg/larva) and Indonesia (LD_{50}= 0.177 µg/larva), respectively.

Emamectin benzoate tolerance level was the highest in the Malaysian FAW population as compared to the other populations from Australia (i.e., Sf20-1, Sf20-4) and Indonesia (Table 6), while tolerance level to Chlorantraniliprole was highest for the Philippines FAW population. With the IPM interim approached being developed by MARDI (see "**7. Key results and discussion:** Current FAW management options being explored and trialled in Southeast Asia: 6. Malaysia" section) where emamectin benzoate and chlorantraniliprole have been recommended to be alternatively used to manage FAW in maize crop of different developmental stages, further understanding of gene flow patterns of Philippines FAW populations to Malaysian FAW populations would be desirable, as introgression of Philippines FAW populations that have elevated Chlorantraniliprole tolerance level with the Malaysian FAW could potentially complicate their management in Malaysia.

A population of FAW from Philippines was used to assess its response to three *Bacillus* species (*B. amyloquefaciens*, *B. thuringiensis*, *B. subtilis*; Table 7). The bioassay results showed that *B. thuringiensis* was the more efficient *Bacillus* species *cf*. *B. amyloquefaciens* and *B. subtillis*, with similar LC_{50} values as the Cry2Ab toxin against the Australia Sf20-1 FAW population and the Australia *S. litura* population (Table 4), although Cry2Ab is generally not regarded as highly effective against FAW. The overall efficacies of these *Bacillus* species in the management of FAW remained unclear and will require further testing, including testing in other SE Asian FAW populations.

These results may suggest that the populations of *S. frugiperda* in a few Southeast Asian countries might have come from different origins, or alternatively from the same origin and have gone through different selection pressure within a short period of time. The various large insecticide and Bt toxin tolerance level differences observed between these SEA and Australian FAW populations were nevertheless unexpected, given that these population were supposedly to have originated from a single founding population from western African regions and established in SEA/Australia within 12-24 months of each other, and large response differences to insecticide are not expected to occur over such a short period of time, as shown in Indian populations of FAW (Kulye et al. 2021).

Table 4: Summary bioassay data involving surface treatment of the diet with Bt toxins and products on *Spodoptera frugiperda* populations from Australia (Queensland and Western Australia) and selected SEA populations from Indonesia and Vietnam. Australian endemic related noctuid species *S. litura* and *Helicoverpa armigera* were also tested to assist with interpretation of bioassay results.

Pesticide	**Populations/ species**	**N**	**Slope**	**LC_{50}**	**95% CI**	**LC_{99}**	**95% CI**	χ^2 (Degrees of Freedom)	***P***	**Toxicity ratio (*H. armigera* = 1)**
Cry1Ac	*H. armigera*	741	1.837 ± 0.126	0.025	0.021 - 0.029	0.465	0.316 - 0.773	31.66 (29)	0.334	-
(µg/cm²)	Sf20-1 (Australia)	504	0.827 ± 0.102	4.34	1.81 - 8.15	80.6	36.85 - 249.17	18.38 (19)	0.497	174
	Sf20-4 (Australia)	575	1.449 ± 0.149	2.48	1.79 - 3.29	85.34	53.32 - 159.68	17.77 (22)	0.720	99
	FAW (Indonesia)	510	0.78 ± 0.11	0.268	0.025 - 0.825	249.25	43.016 - 24199.66	9.06 (5)	0.107	10.7
	FAW (Vietnam)	528	1.33 ± 0.14	5.320	4.220 - 6.575	300.340	168.831 - 667.572	1.99 (5)	0.8505	212.8
	S. litura	501	1.626 ± 0.135	2.99	2.06 - 4.20	80.7	38.60 - 284.54	49.14 (19)	<0.001	120
Cry2Ab	*H. armigera*	476	1.501 ± 0.143	0.049	0.034 -0.068	1.74	0.86 - 6.57	31.834 (18)	0.023	-
(µg/cm²)	Sf20-1 (Australia)	575	1.649 ± 0.136	0.655	0.435 - 0.951	16.88	7.24.- 86.01	86.222 (22)	<0.001	13
	Sf20-4 (Australia)	551	2.201 ± 0.246	0.178	0.138 - 0.221	2.03	1.33 - 4.94	39.076 21	0.010	4
	FAW (Indonesia)	400	1.40 ± 0.17	0.247	0.198 - 0.322	15.711	5.743 - 84.806	0.27 (2)	0.874	5.04
	FAW (Vietnam)	168	1.42 ± 0.26	0.240	0.105 - 0.396	10.450	3.642 - 155.288	0.79 (4)	0.9398	4.89
	S. litura	859	1.197 ± 0.081	0.511	0.335 - 0.734	44.9	20.179 - 154.39	91.946 33	<0.001	10
Cry1F	Sf20-1 (Australia)	576	1.531 ± 0.115	0.025	0.015 - 0.038	0.838	0.365 - 3.949	87.309 (22)	<0.001	-
(µl/cm²)	Sf20-4 (Australia)	575	1.809 ± 0.149	0.021	0.014 - 0.029	0.394	0.211 - 1.138	19.947 (22)	0.586	-
	FAW (Indonesia)	1278	0.89 ± 0.10	0.006	0.002 - 0.012	2.487	0.517 - 100.048	10.64 (5)	0.059	-
	FAW (Vietnam)	179	1.17 ± 0.21	0.030	0.023 - 0.037	2.910	1.307 - 1.633	0.66 (5)	0.9851	-
	S. litura	574	1.524 ± 0.121	0.0088	0.0069 - 0.011	0.294	0.187 - 0.535	58.968 (22)	<0.001	-
VIP3a	*H. armigera*	763	1.486 ± 0.093	0.0062	0.0051 - 0.0075	0.230	0.140 - 0.400	33.738 (30)	0.291	-

(µl/cm²)	Sf20-1 (Australia)	619	1.881 ± 0.225	0.0021	0.0010 - 0.0031	0.049	0.021 - 0.257	68.846 (24)	<0.001	0.152
	Sf20-4 (Australia)	599	2.398 ± 0.212	0.0019	0.0016 - 0.0023	0.018	0.013 - 0.028	20.934 (23)	0.585	0.078
	FAW (Indonesia)	638	1.35 ± 0.25	0.004	0.002 - 0.005	2.572	1.796 - 4.169	4.51 (5)	0.479	0.645
	FAW (Vietnam)	312	1.72 ± 0.22	0.0005	0.0003 - 0.0008	0.012	0.0053 - 0.0740	4.96 (4)	0.2914	0.081
	S. litura	575	1.693 ± 0.129	0.00065	0.0005 - 0.0008	0.015	0.088 - 0.035	39.108 (22)	0.013	0.065
Dipel	*H. armigera*	526	1.832 ± 0.154	2.11	1.59 - 2.72	39.2	22.43 - 92.76	33.878 (20)	0.007	-
(IU/cm²)	Sf20-1	644	1.832 ± 0.154	52.02	36.55 - 70.05	2612.6	1379.89 - 6752.99	29.258 (25)	0.253	24
	Sf20-4	574	1.703 ± 0.153	38.93	23.97 - 56.35	904.1	469.80 - 2779.63	53.767 (22)	0.002	18
	S. litura	788	1.426 ± 0.098	35.96	28.23 - 44.99	1540.8	916.66 - 3110.88	40.916 (31)	0.110	17
XenTari	*H. armigera*	620	1.530 ± 0.136	5.95	4.69 - 7.40	197.2	118.7 - 398.3	23.051 (24)	0.516	-
(DBM/cm²)	Sf20-1	647	2.375 ± 0.289	11.81	8.33 - 15.17	112.7	69.8 - 270.1	39.18 (25)	0.035	2
	Sf20-4	549	1.333 ± 0.180	19.01	9.27 - 29.8	1058.9	379.5 - 10739.7	43.25 (21)	0.003	3
	S. litura	741	2.375 ± 0.289	24.86	17.08 - 31.14	206.9	126.2 - 597.5	47.92 (29)	<0.001	4

Table 5: Summary bioassay data on *Spodoptera frugiperda* populations from Australia and selected SEA populations and Australian *Helicoverpa armigera* (CSIRO GR laboratory line) involving topical application to the insect with contact insecticides.

Toxin	Population/ Species	N	Slope	LC_{50}	95% C.I.	LC_{99}	95%CI	χ^2 (Degrees of Freedom)	*P*	Toxicity ratio (*H. armigera* = 1)
Alpha cypermethrin	*H. armigera*	1328	2.849 ± 0.140	0.0036	0.0032 - 0.0041	0.023	0.018 - 0.032	109.84 (57)	<0.001	-
µg/larvae	Sf20-1 (Australia)	675	2.399 ± 0.154	0.201	0.171 - 0.239	1.88	1.31 - 3.06	43.903 (28)	0.028	56
	Sf20-4 (Australia)	766	2.186 ± 0.132	0.523	0.427 - 0.641	6.06	3.99 - 10.85	67.208 (32)	<0.001	145
	FAW (Indonesia)	1000	2.00 ± 0.14	0.177	0.146 - 0.210	2.572	1.796 - 4.169	3.93 (5)	0.560	49.2
	FAW (Laos)	192	1.75 ± 0.24	1.660	1.192 - 2.259	35.43	18.015 - 109.83	3.01 (5)	0.698	461.1
	FAW (Malaysia)	400	0.73 ± 0.14	0.200	0.087 - 0.413	305.78	34.877- 62744.94	4.00 (5)	0.549	55.6
Methomyl	*H. armigera*	858	0.809 ± 0.059	0.057	0.031 - 0.097	43.12	10.27 - 53.93	135.89 (36)	<0.001	-
µg/larvae	Sf20-1 (Australia)	765	1.064 ± 0.076	0.254	0.177 - 0.356	39.2	15.81 - 156.30	72.309 (32)	<0.001	4
	Sf20-4 (Australia)	631	0.874 ± 0.07	2.96	1.87 - 4.66	1363.7	380.11 - 10950.47	57.160 (26)	<0.001	52
	FAW (Indonesia)	975	1.76 ± 0.12	0.296	0.231 - 0.377	6.231	3.620 - 13.842	7.43 (5)	0.191	5.2

S. frugiperda from Walkamin Queensland and from Kununurra Western Australia are coded Sf20-1 and Sf20-4, respectively. *Helicoverpa armigera* laboratory line is coded as 'GR'. The concentration of each pesticide required to kill 50% and 99% of the test subjects (*H. armigera*, *S. frugiperda*) are given as LC_{50} (50% lethal concentration) and LC_{99} (99% lethal concentration), respectively. 95% confidence intervals (95% CI) for both LC_{50} and LC_{99} are also provided. Sample sizes (N) of *H. armigera* and *S. frugiperda* laboratory culture lines used in the bioassay tests are indicated. P-values (*P*) associated with the χ^2 tests are also provided.

Table 6: Summary bioassay data on Australia and selected SEA populations of *Spodoptera frugiperda* and Australia *Helicoverpa armigera* involving diet incorporation of insecticides.

Pesticide	Population/ Species	N	Slope	LC_{50}	95% CI	LC_{99}	95%CI	χ^2 (Degrees of Freedom)	*P*	Toxicity ratio (*H. armigera* = 1)
Chlorantraniliprole	*H. armigera*	540	3.199 ± 0.234	0.011	0.009 -0.013	0.056	0.038 - 0.109	72.27 (22)	<0.001	-
(µg/ml diet)	Sf20-1	613	2.484 ± 0.191	0.032	0.024 - 0.043	0.28	0.162 - 0.777	99.976 (25)	<0.001	3
	Sf20-4	897	2.065 ± 0.152	0.163	0.132 - 0.201	2.19	1.40 - 4.10	99.895 (38)	<0.001	15
	FAW (Indonesia)	1642	2.26 ± 0.14	0.024	0.156 - 0.032	3.385	1.421 - 30.118	127.13 (5)	<0.001	2.1
	FAW (Malaysia)	400	3.23 ± 0.41	0.030	0.024 - 0.029	0.140	0.114 - 0.175	0.84 (5)	0.974	2.7
	FAW (Philippines)	576	1.71 ± 0.15	0.105	0.070 - 0.144	2.386	1.217 - 7.785	8.6 (5)	0.126	9.5
Indoxacarb	*H. armigera*	653	1.684 ± 0.122	0.054	0.028 - 0.089	1.29	0.49 - 12.59	226.42 (27)	<0.001	-
(µg/ml diet)	Sf20-1	697	2.359 ± 0.159	1.203	1.031 - 1.398	11.66	8.50 - 17.62	34.789 (29)	0.212	22
	Sf20-4	541	1.817 ± 0.130	11.206	9.254 - 13.654	213.85	136.20 - 391.41	24.511 (22)	0.321	208
	FAW (Indonesia)	875	4.69 ± 0.45	8.840	6.773 - 10.768	27.710	19.705 - 60.97	10.56 (4)	0.032	163.7
	FAW (Laos)	192	1.67 ± 0.20	0.643	0.184 -2.053	15.883	3.809 - 4167.37	21.92 (5)	<0.001	11.9
	FAW (Malaysia)	400	3.12 ± 0.49	0.86	n/a - n/a	4.78	1.657 - 0.109	65.11 (5)	<0.001	15.9
Emamectin	*H. armigera*	810	2.465 ± 0.164	0.0107	0.0087 - 0.0131	0.0945	0.065 - 1.607	75.940 (34)	<0.001	-
(µg/ml diet)	Sf20-1	631	2.606 ± 0.175	0.0158	0.013 - 0.019	0.124	0.082 - 2.292	67.73 (26)	<0.001	1
	Sf20-4	720	3.234 ± 0.226	0.0179	0.016 - 0.020	0.094	0.723 - 1.331	40.00 (30)	0.105	2
	FAW (Indonesia)	748	3.76 ± 0.33	0.024	0.156 - 0.032	0.100	0.064 - 0.296	8.44 (3)	0.038	2.2
	FAW (Malaysia)	400	1.00 ± 0.26	0.11	0.000 - 0.793	22.180	1.612 - n/a	13.97 (6)	0.030	10.2
Spinetoram	*H. armigera*	472	1.711 ± 0.173	0.086	0.023 - 0.157	1.977	0.686 - 8.409	154.67 (19)	<0.001	-
(µg/ml diet)	Sf20-1	831	3.220 ± 0.198	0.118	0.101 - 0.137	0.623	0.457 - 0.990	94.168 (35)	<0.001	0.3

Sf20-4	542	4.921 ± 0.489	0.102	0.092 - 0.112	0.301	0.247 - 0.400	13.119 (22)	0.930	1.2
FAW (Indonesia)	1222	3.88 ± 0.27	0.148	0.138 - 0.158	0.589	0.510 - 0.705	1.47 (4)	0.832	1.7

S. frugiperda populations are Sf20-1 from Walkamin Queensland and Sf20-4 from Kununurra Western Australia, *H. armigera* 'GR' belongs to a laboratory line The concentration of each pesticide required to kill 50% and 99% of the test subjects (*H. armigera*, *S. frugiperda*) are given as LC_{50} (50% lethal concentration) and LC_{99} (99% lethal concentration), respectively. 95% confidence intervals (95% CI) for both LC_{50} and LC_{99} are also provided. Sample sizes (N) of *H. armigera*, and the two *S. frugiperda* laboratory culture lines used in the bioassay tests are indicated. P-values (*P*) associated with the χ^2 tests are also provided. n/a indicates 95% CI values could not be calculated by the POLO program.

Table 7: Summary bioassay data on Philippines populations of *Spodoptera frugiperda* involving diet incorporation of selected *Bacillus* spp. toxins as biopesticides.

Pesticide	**Unit**	**N**	**Slope**	**LC_{50}**	**95% CI**	**LC_{99}**	**95%CI**	χ^2 (Degrees of Freedom)	***P***
Bacillus amyloquefaciens	µl/ml	576	0.90 ± 0.11	0.990	0.613 - 1.446	393.420	142.044 -1989.707	72.27 (22)	<0.001
Bacillus thuringiensis	µl/ml	576	0.73 ± 0.10	0.440	0.193 - 1.239	651.410	53.060 - 463274	99.976 (25)	<0.001
Bacillus subtilis	µl/ml	576	0.59 ± 0.11	0.870	0.376 - 1.519	7807.623	1039.048 - 39856.031	99.895 (38)	<0.001

Commercial names of: *B. amyloquefaciens* = MagikGRO; *B. thuringiensis* = Aztron; *B. subtilis* = MagikKILL.

7.3.3 SEA and Australia FAW population genetic characterization

This work represents the most comprehensive population genomic analysis involving native populations from North, Central, and South Americas, and invasive populations from SEA, South Asia, and East Asia, Africa, and Pacific/Australia conducted to-date. The results are in agreement with previous findings of Tay et al. (2022a) that, contrary to the widely accepted and reported spread patterns of the FAW that attributed the rise of the current global invasive populations as the result of a single introduction, genomic analyses identified Asia and SEA as the biosecurity hotspots that have played a significant role in the pest's introduction and spread. Gene flow directionality analysis identified Asian/SE Asian FAW populations as the bridgehead invasive populations of East Africa (i.e., Uganda, Malawi). Unique genomic signatures of Myanmar from China FAW populations provided further support that the spread of this pest was unlikely to be of the same proposed African origin, but instead demonstrated independent introductions even between countries with shared borders.

Detection of FAW in Asia
There is at present a lack of molecular analysis of early FAW samples from SEA, while the earliest FAW from Asia analysed by whole genome sequencing was that from Yunnan China from 2016 (Tay and Gordon 2019; Tay et al. 2022a), supporting that the arrival of FAW in Asia was likely as early as 2016. Surveys of 'grey literature' identified 'presumptions' and 'poor knowledge for FAW' as potential factors for the lack of earlier reports of this pest at least in Vietnam. For example, a perspective piece by Dr Pham Van Lam (Entomologist at PPRI) titled "On time to recognize first potential of *Spodotopera frugiperda* (Smith) (Lepidoptera: Noctuidae) in Vietnam, and its Vietnamese name (2019)" identified that some technicians and famers from various provinces of Vietnam have seen the caterpillars many years before 2019 when the pest was officially confirmed as FAW. The lack of reporting FAW to officials was because the technicians and farmers had presumed that these were maize caterpillars (*Mythimna loreyi*). A Masters thesis submitted to the Agriculture University Vietnam (Vu 2008) provided the first photograph of FAW that was collected from park lawns around Hanoi, with the results also presented at the 3rd National Conference of Ecology and Natural Resources by Nguyen and Vu (2009) in Hanoi, Vietnam.

Detection of FAW in Uganda
While FAW in Uganda was officially recorded as 2017 by FAO, EPPO and CABI, suspect caterpillars collected between July-December 2016 from western and central Uganda were already confirmed as FAW (Otim et al. 2018a). Furthermore, field surveys (Otim et al. 2018b) reported that FAW was first recognized in Uganda since May/June 2016, while farmers from eastern and northern Uganda first reported FAW-like crop damage signs since 2014, similar to the field survey findings by Kalyebi (see Appendix 1; Kalyebi et al. 2022).

Detection of FAW in Australia
From early 2020 the FAW was confirmed in northern Australia in Queensland (Qld), Western Australia (WA), and Northern Territory (NT). While FAW in northern Australia was likely associated with natural migration, population genomic analyses between different Australian populations from Qld, WA, and using the well-established neutral single nucleotide polymorphic (SNP) DNA markers (Tay et al. 2022a) showed limited gene flow between these supposedly related populations (Rane et al. 2022a). This finding contradicted the expected the single introduction and founder event based on reverse trajectory simulation study of Qi et al. (2021), where the Australia's Bamaga founding population was identified as likely originated from Sulawesi and the Moluccas Island of Indonesia, before the population's subsequent spread across Australia. In Australia, the significant genetic differentiation between Western Australian, Northern Territory, Queensland, and New South Wales populations therefore supported multiple pathways into Australia and involved distinct SEA populations (Rane et al. 2022a).

Similar to the findings from Tay et al. (2022a), widespread genome introgression in populations from SEA (i.e., Myanmar, Vietnam, Laos, Malaysia, Philippines), the Far East (i.e., South Korea), and Pacific/Oceania (i.e., Papua New Guinea; Australia) were detected that further supported that recent invasive populations were overwhelmingly hybrids. SNP and concatenated mitochondrial DNA markers also identified multiple introductions in SEA and the Far East populations, while distinct population genomic signatures between Myanmar and China did not support the 'African origin spread' nor the 'Myanmar source population to China' hypotheses (e.g., Lei et al. 2019; Wu et al. 2019; Wang et al. 2020). Instead, the spread patterns identified in Rane et al. (2022a) and Tay et al. (2022a) were most readily and easily explained by anthropogenic-assisted spread, i.e., associated with international trade of live/fresh plants and plant products, and involved countries that served as 'bridgehead populations' (Guillemaud et al. 2011) to enable successful pest establishments in neighbouring countries. Taken as a whole, the project outcome identified Asia as a biosecurity hotspot and a genetic melting pot for FAW, and demonstrated the values of genome analysis approach to disentangle preventable human-assisted pest introductions from unpreventable pest spread via natural migration.

8 Impacts

8.1 Scientific impacts – now and in 5 years

8.1.1 Now

This project represents the first research linking SEA countries with on-going international research effort on FAW genomics to disentangle its global incursion patterns, pathways, and factors that underpinned its perceived rapid spread. Based on analyses of SEA FAW population samples, evidence for multiple introductions of FAW to SEA and between SEA and Asia was supported. Our data suggest the pest has spread also from east-to-west across time. The project therefore provided understanding of the need to bolster biosecurity preparedness, biosecurity protocols, and support for rapid detection of emerging pest threats for the SEA region, by demonstrating signatures of multiple introductions in the FAW. It highlights the need to for conclusions relating to pest spread to be based on sound genomic evidence, and caution the indiscriminate use of partial gene markers (i.e., partial mt*COI* and partial *TPI* genes) to infer introduction pathways. This genomic work has been submitted to the international peer-review scientific journal 'Scientific Reports' (pre-print available from BioRxiv, see Rane et al. 2022b) and has tentatively been accepted pending minor revision. It represents a significant output from the project to build research partnerships between Australia, Uganda, and SE Asian nations. To many co-authors from SE Asia, this manuscript also represents one of the very first genomic work that they have participated to provide the global scientific communities with genome study on the FAW from their countries. The findings from the study will impact on national and regional biosecurity preparedness, and have contributed to the Annual Review of Entomology article on the FAW (Tay et al. 2023) that is currently available as early on-line publication.

• Significant genetic diversity of SEA and Australia FAW populations have been demonstrated that is adding weight to support multiple introductions of the FAW likely underpinned its rapid and widespread detection across its invasive ranges

• Identified SEA, Asia, and East Africa as biosecurity weakness hotspots. With other related *Spodoptera* species capable of also causing significant crop damage that are also likely to be on the move, this work demonstrated the complexity underpinning global pest spread pathways.

• Population genomic results from this project represented the first comprehensive population-wide genome survey and analyses of this highly destructive pest species and set the standards for research especially relating to other emerging transboundary plant pests.

• Significantly increased genome resources for the FAW especially for populations from SEA

• Importantly, results from this study reaffirmed the need for a robust regional biosecurity policy, whereby Australia's biosecurity preparedness and success is intricately linked to its regional neighbours. Investment for Australia's national biosecurity programs will need to also consider how Australia's science can contribute to ASEAN biosecurity RD&E programs through collaborative research programs as demonstrated through this research project.

The project investigated insecticide responses between different FAW populations from SEA and Australia, using standardised bioassay protocols that will facilitate meaningful comparisons of data and results interpretation at the regional level for improved insecticide resistance management. Part of the results involving Australia, PNG and South Korea populations have been peer-reviewed and accepted for publication by the Journal of Economic Entomology (Tay et al. 2022b). Bioassay results and resistance allele frequencies for the remaining SEA populations presented here and in the GRDC final report (Tay et al. 2021a) will enable the wider scientific community to make informed decisions with respect to best management practice involving Bt and chemical insecticidal compounds.

The project developed a metagenomics approach via high throughput sequencing of amplicon for high volume sampling and concurrent characterisation of both C- and R-strain mitochondrial DNA haplotype compositions in FAW populations, and their associated resistance profiles to organophosphate and carbamate insecticides that are conferred by the acetylcholinesterase (ACE-1) resistance gene. The approach can be used to interrogate current population genetic patterns across different countries, thereby allowing genetic monitoring to identify changes to population compositions that could indicate either novel biosecurity breaches, or changes and development of new insecticide resistant populations at landscape scale. This has the potential to support resistance management program (RMP) strategies to monitor for rapid build-up of resistance to specific insecticides or to Bt toxins by developing and using of appropriate DNA markers in the metabarcoding surveys.

• Demonstrated detections of diverse insecticide resistance profiles in different FAW populations in the SEA and Pacific/Oceania regions, and will likely have impact to on-going and future management options of this pest.

The project explores Ugandan farmers' cultural practices for managing FAW, and enable comparisons with current cultural practices across different SE Asian countries to identify common aspects that could be adopted to reduce impact by the pest. It also enables unique cultural practices between Africa (e.g., Uganda) and SE Asian countries to be identified, tested and potentially adopted.

8.1.2 In 5 years time

It is envisaged that the current genomic resources will serve as the foundation for improved RMP and biosecurity preparedness research activities to help farmers in SEA to better managed the FAW. We also envisaged that the whole genome sequencing analysis approach developed in this study will be further deployed for on-going surveys of other invasive FAW populations to detect novel pest introduction pathways and regional gene flow patterns between established populations, with the outcome helping to minimise future accidental introductions of novel genetic traits to the current established populations. Importantly, we envisaged that the genome signatures of these current pest populations will facilitate research into better understanding how pest species such as the FAW respond and adapt to changing climate conditions. The current project output to understand cultural pest management practices across SE Asia and Africa is expected to play a central role in the advancement of alternative cultural management and genetic control solutions for this pest.

8.2 Capacity impacts – now and in 5 years

Now

The project fosters scientific collaboration between seven of the ten SEA nations and Australia and provides exposure opportunities for SEA partner countries to participate in genomics research of the high profile exotic invasive insect pest, S. frugiperda, at the regional level. It also enables the partner countries to develop skillsets and build capacity in insecticide bioassays that will be relevant to future emerging pest threats. The project facilitated linkages between SEA partners and the ASEAN FAW Action Plan program to improve smallholder productivity, profitability, training, and environmental sustainability especially in aspects relating to integrated pest management of FAW.

Specific capacity impact areas include:

1. Partner countries have increased their ability to carry out insecticide and/or Bt bioassay experiments on FAW. This is especially significant as at the commencement of the project partners from e.g., Laos PDR and Philippines, have never conducted bioassay experiments involving insecticides and/or Bt toxins.

2. Partner countries have been given the opportunity to be exposed to genome-based research in transboundary plant pest using the FAW as a case study.

3. Partner countries have successfully reared and maintained FAW colonies in laboratory settings to enable experiments to be conducted, this included developing country-specific FAW rearing protocols.

4. Interactions between researchers from partner countries enabled experts with unique skill sets (e.g., experts in cultural management practice; insecticide bioassays) to be identified and to form collaborations to build regional capacity.

The capacity build by the project partners from SEA and Australia will play an important role in future regional agricultural research activities. We envisage that in 5 years' time, partner countries will utilise skills relating to bioassays to conduct resistance management research on other insect pest systems as well as to have confidence in monitoring the development of novel resistances in the FAW. Through this project, partner countries will increase their overall participation in future pest genomic research activities, building on the understanding gained from this current research to better utilise genome resources to address basic and applied agricultural research questions. Importantly, some partners will emerge to become regional leaders with significant research skills and knowledge capacity to lead other national and regional research programmes on the FAW, including to effectively develop and utilise their bioassay skills to test promising endemic biocontrol agents such as on new isolates of *Bacillus thuringiensis* for FAW management, on novel entomopathogenic fungi, and on local nucleopoloyhedrosis viruses (NPV) to further refine regional IPM toolkit to manage the FAW.

8.3 Community impacts – now and in 5 years

At the community level, the FAW has caused significant economic losses and negatively impacted on the mental health, food security, and economic security of small-scale farmers in the SEA region. Due to the short period of time relating to the region-wide pest outbreak, missed opportunities at early detections, and the resulting high negative economic impact, there has been insufficient time to engage farmers in educational programs and in developing regional-specific RMP and IPM resources, such as on responsible usage of insecticidal compounds and alternative management strategies involving eco-friendly bioagents. This has led to excessive applications both in quantity and in frequency of chemical spray to contaminate the farming landscape, environment, and negatively impacted the ecosystems especially on beneficial insects and other predators of FAW. The sudden outbreak of this highly damaging insect pest also led to increased anxiety in the farming communities due to the associated significant crop losses experienced in the early stage.

Funding to support a regional scale research involving some of the poorest nations in SEA (and Africa) therefore help raised the community spirit and to remind the FAW-impacted communities that international support was working to find solutions to a common challenge. The funding targeted important management issues relating to the sustainability of prolonged usage of different insecticidal compounds and provided local researchers exposure and build confidence to would-be leaders and problem solvers for their own communities.

Going forward in 5 years' time, impact on local communities could include farmers and growers' ability to confidently manage the FAW using knowledge developed by local and regional researchers who have been participants of this ACIAR/GRDC co-funded project. The bioassay techniques and knowledge gained from participating in the project will contribute to reduced environmental pollution due to excess frequency and incorrectly applied amount of insecticides; with the local communities further benefited from efficient cultural pest management solutions developed, identified, and refined from this project.

8.3.1 Economic impacts

The potential economic costs of mobile pests such as the FAW are high globally including for growers from the pest's native American geographic ranges, and especially also for smallholder farmers from the recent invasive ranges (i.e., sub-Saharan Africa, Asia, SE Asia, the Pacific), but also for large scale growers from countries such as Australia. For example, in the ASEAN region, direct yield loss (e.g., conservative estimate of 10% crop damage in maize crop only) caused by larval feeding and indirect costs, e.g., from buyers forced to import maize, was estimated to be US$884 million (ASEAN FAW Action Plan; accessed 12-Oct, 2021); and excluding management costs associated with pesticide applications (some of which may not be effective), the risk of secondary pest outbreaks and resistance development in both target pests and non-target species. Our work addresses these impacts by making sure that pesticide application advice is tailored to both the farmers needs and the pest population traits in a region. Furthermore, the project identified widespread regional biosecurity protocol and preparedness gaps based on genomic evidence to inform relevant policy practitioners, which will have flow-on effect for future detection and management of emerging pest threats to protect and benefit regional economic livelihood especially for small-scale farmers.

Specific economic impact output from the project included:

• Identified the purchase of insecticides by small scale farmers in Africa and Asia as a major economic burden

• Identify the lack of knowledge on correct usage of insecticides to control the FAW as a factor to negatively impact on the livelihood of these farmers

• identified the widespread and excessive usage of insecticides could lead to secondary pest outbreak to further negatively impact on farmers' livelihood

In 5 years time

We anticipate there will be significantly less economic impact from the FAW especially due to improved insecticide usage and implementation of knowledge generated from e,.g., the ASEAN project teams, including being able to undertake appropriate bioassay experiments to assist with developing regional-specific management strategies. We also anticipate that insecticide efficacies will be maintained due to responsible insecticide usage to ensure crop production costs are not unnecessarily increased. Importantly, we anticipate there to be regional-scale adoption of cultural pest management practices, and improvement and up-date of relevant IPM and RPM to help lower economic impact from FAW.

8.3.2 Social impacts

Monitoring for development of insecticide resistance can prolong current socially accepted FAW management practices involving chemical and Bt insecticidal compounds, failure of which could negatively impact social resilience. If products that are sold to control a certain pest fail to do so, this can create distrust with agri-input sellers more broadly. Even if the reasons behind the failure have little to do with incorrect advice or application. However, if resistance is a documented problem in a local pest population, and this is known by agri-input sellers they can suggest alternative management actions.

The project has helped to promote the visibility of partner country researchers as leaders in their scientific field who can address applied problems of relevance to their local farmers. The activities undertaken have encouraged participation between researchers and farmers in addressing a significant concern and challenge in the community. We hope there will be opportunities for the researchers in each country to communicate the project findings to local groups (extension officers, agri-input sellers, growers groups) and regionally via the ASEAN FAW Action Plan group.

8.3.3 Environmental impacts

Exploring alternative pest management strategies such as cultural practices that can be integrated with pesticide use can reduce environmental pollution caused by pesticide movement into soils and waterways.

The bioassay experiments conducted in this project helped to inform our understanding of the efficacy of trialled insecticides and identify optimal application rates that could be extended to field trials. Access to this information by small-holder farmers, agri-input sellers, extension officers, and spray applicators will help to reduce the chance of excessive application rates.

This research identifies insecticides that are unlikely to effectively control FAW. By minimising the use of these insecticides we are reducing the risk of negative impacts on beneficial invertebrates (predators and parasitic wasps) and thereby reducing the risk of secondary pest outbreaks. Pollination services by invertebrate pollinators in farming systems will also be maintained.

8.4 Communication and dissemination activities

Output from this project included multiple cutting-edge scientific peer-review papers, industry reports, newsletter up-dates on project, news interviews, and contributions to webinars and symposiums.

Peer-reviewed and under review scientific papers generated from this project are provided in section 10.2

Industry Reports

Two final industry reports were submitted to the GRDC

1. Tay WT, James B, Walsh T. (2021a) GRDC Final report on Australian bioassays. Prevention and preparedness for fall armyworm (*Spodoptera frugiperda*) – Output 2. Project code: CSP2003-008RTX. Date Submitted: 22 June, 2021. 31pp.

2. Tay WT, Rane R, James B, Dao TH, Nguyen VL, Khin TN, Amalin D, Chittarath K, Faheem M, Sivapragasam A, Trisyono YA, Sathya K, Walsh T. (2021b) GRDC Final report on Southeast Asian and Australian FAW population genomics for biosecurity preparedness. Prevention and preparedness for fall armyworm (*Spodoptera frugiperda*) – Output 2. Project code: CSP2003-008RTX. Date Submitted: 02 July, 2021. 35pp.

Newsletters up-dates

1. Tay WT, Dao TH, James W, Rane R, Nguyen VL, Walsh T. (2021) Transforming the fall armyworm insecticide-resistance management in South-East Asia. November 2021 ACIAR in Vietnam Newsletter. Pp32-34.

News interviews

1. Project to combat fall armyworm in Australia and South East Asia. 9 July 2020. News Release <https://www.csiro.au/en/news/news-releases/2020/project-to-combat-fall-armyworm-in-australia-and-south-east-asia>

2. 1. Project to combat fall armyworm in Australia and South East Asia. Australian Grain, July-August 2020. pp37-38. <https://www.greenmountpress.com.au/download.php?MagID=1&pages=39,40>

2. Two threats highlighted in the shadow of COVID-19. 17 September 2020. ACIAR News and Media Blogs. <https://www.aciar.gov.au/media-search/blogs/two-threats-highlighted-shadow-covid-19>

3. Managing fall armyworm in Australia and South-East Asia. ACIAR News, 10 July 2020. <https://www.aciar.gov.au/media-search/news/managing-fall-armyworm-australia-and-south-east-asia>

4. CSIRO Leads Armyworm Project. Southburnett.com.au, 13 July 2020. <https://southburnett.com.au/news2/2020/07/13/csiro-leads-armyworm-project/>

5. International, cross-industry project to combat fall armyworm. Spotlight on Cotton R&D, Spring 2020. Pp12-13 <https://www.crdc.com.au/sites/default/files/pdf/Spotlight%20Spring%202020.pdf>

6. Insecticide differences for fall armyworm. Australian Cane Farmers, 4 May 2021. <https://www.acfa.com.au/?cat=-1>

7. Differences in insecticide sensitivity shown in fall armyworm, Mallee Farmers, April 2021, page 5 <https://d3pbdxdl8c65wb.cloudfront.net/n/493/2021/Apr/22/0354/Friday,%20April%2023,%202021.pdf>

8. Differences in insecticide sensitivity shown in fall armyworm. getINDUSTRY. 29 March 2021. <http://getindustry.com.au/2021/03/29/differences-in-insecticide-sensitivity-shown-in-fall-armyworm/>

9. Differences in insecticide sensitivity shown in fall armyworm. Country News, 4 April 2021 <https://www.countrynews.com.au/cropping/2021/04/04/4071038/differences-in-insecticide-sensitivity-shown-in-fall-armyworm/>

10. FAW's genetics and insecticide sensitivities explored to develop pest management plans. GRDC GroundCover, 1 August 2020.

<https://groundcover.grdc.com.au/weeds-pests-diseases/biosecurity/faws-genetics-and-insecticide-sensitivities-explored-to-develop-pest-management-plans?msdynttrid=8KrL1wXi6V1Oi2G5FS97mdRZm3qlC0OdEYEperjyR_o>

Webinars and Symposiums

1. Tay WT. FAW Preparedness & Management Solutions – Academic Partnerships. Food and Agriculture Organization of the United Nations. International Plant Protection Convention, 31 Dec, 2020. <https://assets.ippc.int/static/media/files/publication/en/2020/12/Interactions_with_countries_and_partners_ACADEMIA_WeetekTAY.pdf>
2. Tay WT. Effective regional biosecurity for a changing world. Risk and impacts of transboundary plant pests for Australia and Australia's near-neighbour. ACIAR-AARES Pre-conference Symposium at AARES2021: Effective regional biosecurity for a changing world. 8th February 2021, CSIRO Discovery Centre Theatre, Black Mountain, Canberra, ACT, Australia.
3. Tay WT. Insecticide resistance – current R&D and gaps. Plant Biosecurity Research Initiative (PBRI) Fall Armyworm Workshop. 27 April, 2021
4. Tay WT. Biosecurity preparedness & bioassays of FAW in Australia – Current R&D and gaps. CSIRO Monday Cotton Science Series, Black Mountain Laboratories, ACT, Australia. 21 June 2021.
5. Tay WT, James B, Trisyono YA, Aryuwandari VEF, Nguyen VL, Dao TH, Walsh TK. Report Back: Resistance programme CSIRO SEA Partners Research. ASEAN FAW Action Plan webinar 24 Feb, 2022.
6. Rane R, Walsh Tk, Gordon KHJ, Downes S, Macfadyen S, Tay WT. East and West: working together to disentangle FAW global introduction pathways. 2nd Australian Biosecurity Symposium. 3-5 May, 2022. RACB Royal Pines Resort, Gold Coast, Queensland, Australia.

7. Tay WT. Fall armyworm management and biosecurity risks. CABI South East Asia Seminar, Post-Harvest Complex, MARDI Headquarters, Serdang, Malaysia. 25-July, 2022.
8. Y. Andi Trisyono, Walsh TK, Rane RV, Gordon KHJ, Dao TH, Nguyen VL, Khin TN, Chittarath K, Amalin D, Faheem M, Thanarajoo SS, Sivapragasam A, Khay S, Aryuwandari V, Kalyebi A, Otim MH, Tay WT. Response of FAW population in Southeast Asia to several insecticides and *Bt* Toxin. ASEAN FAW Work Plan Part 2 webinar series on Climate change, resistance and genomics with a focus on fall armyworm. 6-September 2022
9. Tay WT, Walsh TK, Rane RV, Gordon KHJ, Dao TH, Nguyen VL, Khin TN, Chittarath K, Amalin D, Faheem M, Thanarajoo SS, Sivapragasam A, Trisyono YA, Khay S, Aryuwandari V, Kalyebi A, Otim MH. Next steps: what does the genomics research tell us about key areas for further research and what are the knowledge gaps we might address. ASEAN FAW Work Plan - Part 2 webinar series on Climate change, resistance and genomics with a focus on fall armyworm. 6-September 2022

9 Conclusions and recommendations

Despite the challenges faced by all project partners from Australia, Uganda, and from the SE Asian nations (Indonesia, Malaysia, Myanmar, Cambodia, Vietnam, Laos DPR, Philippines) from geological event (i.e., the eruption of the Taal volcano), climate events (cyclones, typhoons, monsoon) that resulted in significant floodings, political unrests in Myanmar, and the SARS-CoV pandemic related health and social disruptions, this project has nevertheless produced significant output to benefit farmers and local grower communities, and to impact national, regional and international policy practitioners relating to pest management and biosecurity. It highlights the need of regional robust biosecurity policies due to increased volumes of international trade between countries. The project showcases small-scale farmer resilience especially in Africa and Southeast Asia, and their resourcefulness in finding alternative cultural pest management practice solutions for the FAW. Research areas specifically relating to exploring alternative management solutions for the FAW in SE Asia and in Africa have been identified, while the use of genomic resources to support RMP and biosecurity preparedness was demonstrated.

9.1 Conclusions

Cultural practices including the push-pull strategy that appeared effective as reported by Ugandan farmers have not been extensively practiced in the SE Asia region despite being promoted by various SE Asian government agencies (e.g., Malaysia). Adoption of the push-pull cultural practice may require re-designing of cropping landscape in some SE Asian countries where the planting areas are larger than in e.g., Uganda. There is a general need of taxonomical support to assist with identifying beneficial insects especially of parasitoids species. While confirmation of novel species could be supplemented with molecular diagnostics, there is nevertheless a need to increase local researchers' skills and knowledge on molecular diagnostics, and may require training courses that could be provided by Australia, e.g., by CSIRO.

Bioassays involving insecticidal compounds and Bt toxins while attempted by different partner countries, there were nevertheless gaps that could be better addressed under less challenging circumstances. These include for all partner countries to agree on testing on agreed set of insecticides and Bt toxins. While partners expressed the desired to better utilise endemic biocontrol agents such as entomophathogenic fungi and NPVs, these resources were nevertheless not addressed due to time limitation. Biopesticides such as Neem oil and other local botanical extracts could be further explored to complement existing management practices or be incorporated into new cultural management solutions.

An all-partner peer-reviewed manuscript (Rane et al. 2022b) is in the process of being revised to address minor comments raised by the reviewers for the international scientific journal Scientific Reports. This study reports on the overall population genomic findings of this destructive pest and highlighted the challenge relating to sharing of biological specimens to address common concerns. Bioassay results and whole genome sequence data to assess resistance allele frequencies for FAW from Australia, Papua New Guinea, and South Korea have been accepted for publication by the Journal of Economic Entomology (Tay et al. 2022b), while the population genomic findings from Rane et al. (2022a) contributed significantly to the high impact Annual Review of Entomology article by Tay et al. (2023). The approach for publishing the bioassay study by Tay et al. (2022b) can be adopted for publishing the bioassay findings from the SE Asian FAW populations to further impact on regional scientific output and to forester collaboration between research partners.

9.2 Recommendations

• While PPRI (Vietnam), UGM (Indonesia), and DLSU (Philippines) expressed the desire to involve graduate students and/or Research Scientists to participate in the genomic analysis to infer population genomic structure and/or resistance gene characterisation, however, this has not eventuated due to border closure and/or enhanced restriction (i.e., prolonged quarantine period) relating to hosting visitors.

• While it was planned for CSIRO to share analysis procedures with SEA partners that would like to participate in the genomic analyses, however this was found to be a difficult task that would require extensive in-person bioinformatic and evolutionary genomics training. A research centre in SEA such as the ASEAN Bioprotection Research Centre (ABRC; funded by DFAT and lead by CSIRO) that is currently being co-designed and the associated feasibility study undertaken, a dedicated training course designed to address specifically population genomic questions, and/or development of targeted web-based solutions could assist with delivering this project objective in the future.

• While various SEA partners (e.g., PPC Lao PDR; DLSU Philippines; CARDI, Cambodia) also expressed the desire to learn to handle Sanger sequencing trace files and this will may be via the basic sequence analysis course as developed and delivered by CSIRO (Tay WT, Macfadyen S) and Natural History Museum London (Polaszek A) at NaCRRI Uganda in 2018, based on software freely available from public domain (e.g., Staden DNA sequencing analysis and assembly software; CLC Sequence Viewer), this was nevertheless not attempted due to time availability of project partners and the difficulties of generating personal sanger sequence dataset to be used for this purpose. Through this project we identified that there remained significant knowledge and molecular technique gaps to enable wide adoption of molecular tools across SEA.

Future opportunities identified by all project partners during the final project meeting held in Singapore on 23-July, 2022 included:

1. To identify new bioagents/biopesticides and parasitoids for better IPM options of FAW
    a. Include cage and field trials using biopesticides/bioagents
2. Testing pesticide resistance in different FAW populations from different countries
3. Identify three main findings from the current work that needed further investigation
    a. Further develop the ‘Push-Pull’ technique to deliver multiple benefits to the farmers
    b. Further investigate the ‘food-spray’ technology demonstrated in Vietnam to confirm efficacies in pest management
    c. To investigate maize variety’s tolerance to FAW across the pest’s invasive range
4. Genomic analyses to understand the impact of climate change on the pest and farm resilience
    a. Increase genomic studies of FAW populations across the invasive range
    b. Workshop on genomic analyses and easy-to-use genomic analytical toolset
5. Cost Benefit analysis of the future opportunities listed above
6. Better understanding and support for pre- and post-border risk assessment of invasive species
7. The need for national diagnostic protocols
8. On-going monitoring and surveillance at regional scale, and how to engage extension personnel
9. Develop a well-integrated data management network to support automation and data modelling
10. Farmer education and support including on effective mobile technology support to manage the FAW

Crucial to developing and realising the above future opportunities are factors such as: (i) funding sources, (ii) human resources and expertise, (iii) equipment and infrastructure especially in the SEA region, (iv) the need of coordination among stakeholders, and (v) disconnect and low adaptation from the industry.

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10.2 List of publications produced by project

1. Tay WT, Kuniata L, James W, Walsh TK (2022) Confirmation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Papua New Guinea by molecular diagnostics of mitochondrial DNA COI gene. BioInvasions Records (revised and resubmitted 20-Aug, 2022).
2. Tay WT, Rane RV, James W, Gordon KHJ, Downes S, Kim J, Kuniata L, Walsh TK (2022) Resistance bioassays and allele characterisation inform analysis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) introduction pathways in Asia and Australia. Journal of Economic Entomology (in press). DOI: 10.1093/jee/toac151
3. Rane RV, Walsh T, Lenancker P, Gock A, Dao TH, Nguyen VL, Khin TN, Amalin D, Chittarath K, Faheem M, Sivapragasam A, Thanarajoo SS, Trisyono YA, Khay S, Kim J, Kuniata L, Powell K, Kalyebi A, Otim M, Gordon K, Tay WT. (2022) Complex multiple introductions drive fall armyworm invasions into Asia and Australia. Scientific Reports doi 10.1038/s41598-023-27501-x, Available from bioRxiv 2022.06.11.495773; doi: https://doi.org/10.1101/2022.06.11.495773
4. Kalyebi A (2021) SEA FAW Project – Documentation of farmer practices that have been useful in Africa for managing the fall armyworm (Spodoptera frugiperda): A case study from Uganda. GRDC Prevention and preparedness for fall armyworm (*Spodoptera frugiperda*) – Output 2. Project code: CSP2003-008RTX. 31pp. Date Submitted: 01 June, 2021
5. Kalyebi A, Walsh T, Tay WT. (2022) Farmer perception of impacts of *Spodoptera frugiperda* and transferability of management practices in Uganda. CABI Agriculture and Bioscience. In Prep.
6. Tay WT, Walsh T. (2021c) Characterisation of *Spodoptera frugiperda* (fall armyworm) populations in South-East Asia and Northern Australia (co-funded with GRDC). CROP2020144. Annual report. Date submitted 10-October, 2021. 23pp.

11 Appendixes

11.1 Appendix 1: Final Report on Ugandan Farmer Field Surveys and *Spodoptera frugiperda* (FAW) Management Practices

ACIAR Report # CROP/2020/144

GRDC Prevention and preparedness for fall armyworm (*Spodoptera frugiperda*) – Output 2 (REFERENCE: CSP2003-008RTX)

SEA FAW PROJECT- DOCUMENTATION OF FARMER PRACTICES THAT HAVE BEEN USEFUL IN AFRICA FOR MANAGING THE FALL ARMYWORM (*Spodoptera frugiperda*): A case study from Uganda.

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Summary

This study reports the perceptions and economic impact of the FAW in Africa (Uganda in particular) and documents measures and alternative management practices used by farmers to control the FAW since its invasion of the continent. While FAW was officially reported in Uganda in 2016, farmers noticed the FAW symptoms and damage at different times the earliest being 2013 in Namutumba district and 2014 in Kamuli district with latest reports recorded in 2020 for some areas.

Most farmers have recorded yield losses in the magnitude of 25-50%. The majority of farmers (64% in Kamuli, 81.6% in Namutumba) therefore considered the FAW to still be a very serious challenge to maize production in their localities. About 24% of the farmers in Kamuli district and 87.8% of the farmers in Namutumba could correctly identify the FAW by its appearance.

To manage the FAW, 84% of the respondents in Kamuli and 89.8% of those in Namutumba districts reported to use mostly chemical control methods. Other methods included the cultural control by regular weeding and hand picking. The use of pheromone and biological control methods to manage FAW was not reported but the use of biological extracts (Pepper, tobacco, aloe-vera, lantana, sisal) was evidently common. About 4% of farmers in both Kamuli and Namutumba reportedly took no action against FAW.

The majority of the farmers (64% in Kamuli, 59.2% in Namutumba) reportedly had between 10-30 years' experience in growing maize with a scale of production of either medium (50% in Kamuli, 53.1% in Namutumba) or small (48% in Kamuli, 44.9% in Namutumba), and given their level of education and

experience advocated for an area-wide approach as one of the best alternatives to completely manage this invasive pest.

11.1.1 Introduction

The Fall armyworm (FAW) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) that has established across the African continent is a highly polyphagous and destructive pest of many crops. A major pest of maize, the FAW is known to have a wide host range and is reported to feed on more than 100 plant species that include cereals, legumes, cotton, potato, banana, vegetables and grasses (Pashley, 1988; Luttrell and Mink, 1999). The larval stage of the FAW is the most devastating stage. Native to the North, Central, and South Americas, the FAW was first reported on the continent of Africa in 2016 from West Africa (Goergen et al. 2016) but by 2018, it was reported also in at least 44 African countries that included all of the sub-Saharan Africa (Rwomushana et al. 2018; Uzayisenga et al. 2018) and Egypt in northern Africa (e.g., IPPC 2019a), as well as the Middle East and the Indian sub-continent. The FAW has also spread further to the Near East and into Asian/Southeast Asian countries including India (Ganiger et al. 2018; Sharanabassappa et al. 2018; EPPO 2019), China (IPPCb 2019a; Shrikanth 2019), Japan, Myanmar, Vietnam (Hang et al. 2019; IPPC 2019c), Malaysia, Indonesia, Papua New Guinea, Philippines (Navasero et al. 2019), prior to being detected in Australia by February 2020 (Hort innovation 2020).

In Africa, FAW consumes a wide variety of cereal crops, particularly maize which is the major staple grown by most farmers (FAOSTAT 2016). The FAW is currently a threat to food security and incomes, and threatens the livelihoods of millions of people as it has led to increased production costs and hinders trade because of quarantines imposed on produce from affected countries. Since its establishment on the continent of Africa, the FAW continues to cause severe destruction to crops that support the livelihoods of many farmers due to the variety of host plants available and the favourable environment and climatic conditions (Goergen et al. 2016). The FAW causes especially severe damage to maize, feeding on virtually all parts of the plant that result in total crop failure (De Almeida Sarmento et al. 2002). Potential yield reduction due to the FAW pest in Africa has been estimated in the range from 8.3 to 20.6 tonnes per year where no control measures have been applied (Abrahams et al. 2017).

In Uganda, maize is one of the most important cereal crops and smallholder farmers usually engage in maize growing for food and also as a cash crop. Maize is an important export crop that earns the country foreign exchange. It is therefore an important food and security crop that supports the livelihood of millions of small-scale farmers. Over the years, production of maize increased from 2.8 million metric tonnes in 2015 to 4 million metric tonnes in 2017 (MAAIF 2018) as a result of the increased demand for maize and other products, and the favourable climate that enables two cropping seasons in a year. Production of maize in Uganda was also stepped up to supply its neighbouring countries (Kenya, Tanzania, Rwanda, South Sudan, D.R. Congo) where it is a staple food for human consumption (e.g., with Kenya having an annual demand of 60,000 metric tonnes).

11.1.2 Challenges and opportunities

Maize yields in Uganda (i.e., production, productivity and quality) have remained relatively low (2.2 to 2.5 metric tonnes/hectare) compared to the potential of 8 metric tonnes/ha because of several biotic and abiotic factors, namely, pests and diseases and declining soil fertility, drought stress and inadequate extension services (MAAIF 2018). The quality standards are also generally low with high post-harvest losses during transportation, storage and processing, and aflatoxin contaminations make it uncompetitive for regional markets.

Maize is attacked by numerous pests and diseases during the growing cycle, with infestation level and incidence dependent on weather factors, soil conditions, interactions with other arthropod species, and the level of resistance/susceptibility of the maize varieties. Pests of maize include the new invasive FAW, cereal stemborers/the maize stalk borer *Buseola fusca*, the spotted stem borer *Chilo partellus,* the African pink borer *Sesamia calamistis*, cutworms, termites, maize weevils etc. Traditionally in Uganda, the main field pests of maize have comprised *C. partellus* and *B. fusca* being the two most damaging (Matama-Kauma et al. 2007). Elsewhere in Africa, *C. partellus* was the most important lepidopteran pest (e.g., Sohati et al. 2007; Cugala and Omwega 2001; Wale et al. 2006). However, a lot of efforts especially by biological control helped to reduce the impact caused by these pests to very low levels (Sohati et al. 2007; Matama-Kauma et al. 2001; Wale et al. 2006).

Currently, the FAW is the major pest of maize in Uganda causing heavy damage as they feed heavily on shoots and growing points. The FAW was confirmed via molecular diagnostics in Uganda from field-collected samples in May/June 2016 (Otim et al. 2018). Despite an increase in maize production from 85% to 92% from 2014 to 2015, there was a drastic reduction in production in 2016 and 2017 to 81.5% which was attributed, in part, to both FAW (33%) and drought (23%) (NARO-ATAAS 2018). In Malawi, 382,000 hectares of maize, sorghum and millet were affected by the FAW by February of 2020 impacting over 1 million households (MoAIWD 2020). In Zambia, surveys showed the pest had spread in all major agro-ecological zones with potential annual economic losses estimated at US$ 159 million (Rwomushana et al. 2018).

Since its invasion, the major form of control advocated in African countries has been the use of insecticides. Because of the devastating effect of the invasive pest, and based on infestation rates, governments prioritised pesticide usage as an immediate response and procured pesticides for distribution to farmers (MoAIWD 2020; MAAIF 2018). Governments raised awareness about the pest and provided some support to farmers with chemical insecticides. However, reliance on use of chemical insecticides comes with its own challenges. In Ethiopia and Kenya, more than 50% of the maize growers that applied chemical pesticides for FAW control reported only marginal control efficacy or were completely ineffective (Kumela et al. 2019). The chemical pesticides are not only ineffective but expensive and pose serious detrimental effects to humans, biodiversity and the environment. Without adequate knowledge on the ecology and biology of the pest and without sound knowledge on the timing, method and frequency of application, dilution rates, and stage of insect's life cycle to spray, farmers are bound to misuse, overuse and un-necessarily use pesticides which not only increases production costs but also poses risks to consumers and the environment.

There is evidence that the over-use, misuse, or un-necessary use of synthetic insecticides particularly carbamates, pyrethroids and organophosphates (common pesticides available to African smallholder farmers) against the FAW can promote the development of resistance (Yu 1991; Carvarlho et al. 2013; Gutireez-Moreno et al. 2019; Zhang et al. 2020). The integration of other non-chemical practices such as cultural, mechanical, physical and biological options is thus important for sustainable management of the FAW.

11.1.3 Objectives of study

To compliment the on-going study investigating management of the FAW in Southeast Asia, this study sought to understand what measures and alternative management practices have been used by farmers in Africa to control the FAW since its invasion beyond what is available in the literature. Uganda was selected to host this study, being one of the countries in Africa where the FAW invaded early on. The overall objective of this study was therefore to document practices that have been useful in Africa to manage the FAW by farmers. The specific objectives of this study included:

(i) Establish from farmers' perspective the time of arrival of the FAW
(ii) Investigate the economic impact (yield and income) of FAW to maize farmers
(iii) Establish the farmers' current perception of the status of the FAW since its invasion
(iv) Record alternative practices used by farmers to manage the FAW

11.1.4 Materials and methods

We designed a 5-page questionnaire (Appendix I) to provide focus and guide us on achieving the set objectives aimed at assessing the farmers' socio-economic profiles, focussing around maize production. Such questions included those on gender and education levels, membership to farmer organisations, economic profile of farmers based on maize, awareness of FAW and its identification, knowledge of FAW damage and economic impact, ability of farmers to identify FAW pests, management practices used in FAW control, means of agricultural information exchange amongst farmers, and what in general are ways- forward to manage the FAW challenge in their respective areas.

Farmer economic profile based on maize mainly focussed on yield of maize per acre before and after FAW arrived, and also assessed farmers understanding of the impact of FAW based on whether they consistently and logically responded to questions on effects on income, production, yield and production costs. The maize production profile entailed establishing the varieties of maize grown before and after FAW invasion, the source of seed used during plantings, the farmers experience of maize growing in years, scale of production whether small, medium or large and the type of cropping system under maize (whether organic or inorganic, monoculture or mixed). The scale of production was regarded small if the famer planted less than 2 ha; medium if the farmer planted more than 2 ha until 9 ha, and it was large if the farmer had more than 10 ha.

A question was also designed to capture when farmers noticed the FAW, its symptoms and/or damage for the very first time in their fields, and also if they noticed any method they tried to use against the FAW fail. The questionnaire was administered to the farmers (in form of an interview) (Fig. 1) to help understand the key questions that underpin the study objectives. The interviews were conducted in the local language of the area (i.e., Lusoga, for those that did not have good command of the English language) during the face-to-face interactions. In other areas, a mix of both Lusoga and English was used for clarity. We included both small- and large-scale farmers based on the acreage of maize planted in the last two seasons. A quick assessment was also made by the farmers (Fig. 2) and interviewers (Fig.3) to ascertain the presence of the FAW in the farmers' field, and also to assess of the farmers' knowledge of the FAW through identification/recognition of its stages, symptoms or damage. Using a pictorial chart of insect larval developmental stages (see Appendix II), farmers were also asked to identify which stages they had seen commonly within their fields. This moment was also used to sensitize the farmers about the different stages of the FAW life cycle (some of which they had not seen before but were important) for proper management of the FAW.

The investigations were carried out in November 2020 in Kamuli and Namutumba, which are two maize growing districts located in eastern Uganda (Fig. 4a). Within each district, focus was put on sub-counties (administrative units) where maize growing was prominent- a selection of this was made purposively prior to the investigation with the help of extension agents within each district. In a sub-country, farmers were randomly picked from among recognised farmers based on sub-country records. A total of 50 farmers were interviewed per district.

Data on maize yield/production was obtained on two seasons before and after fall armyworm invasion to understand the challenges attributed to fall armyworm attack. The data was also based on recall of estimates by the farmer. Such quantification of yield estimates provided farmers perceptions of the damage posed by the fall armyworm.

Data analysis

Descriptive statistical analyses were undertaken to calculate the frequencies, means, and percentages where appropriate, and when necessary, differences between variables of interest determined by use of chi-square and ANOVA tests.

Fig. 1: Conducting an interview-with a farmer in (a) Kamuli district, (b) Namutumba district

Fig. 2: Evaluating farmers' ability to locate and identify FAW larvae, damage and symptoms

Fig. 3: Scouting for FAW in farmers' fields in Namutumba district

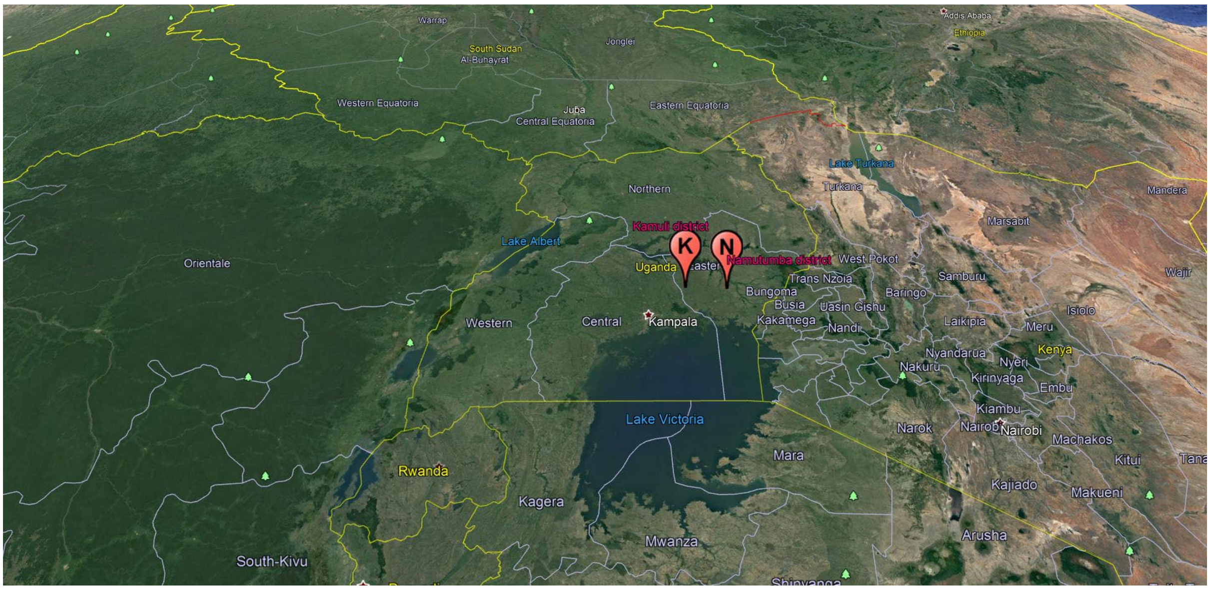

Fig 4a. Map of Uganda showing the location of the two districts Kamuli (K) and Namutumba (N) where surveys were carried out.

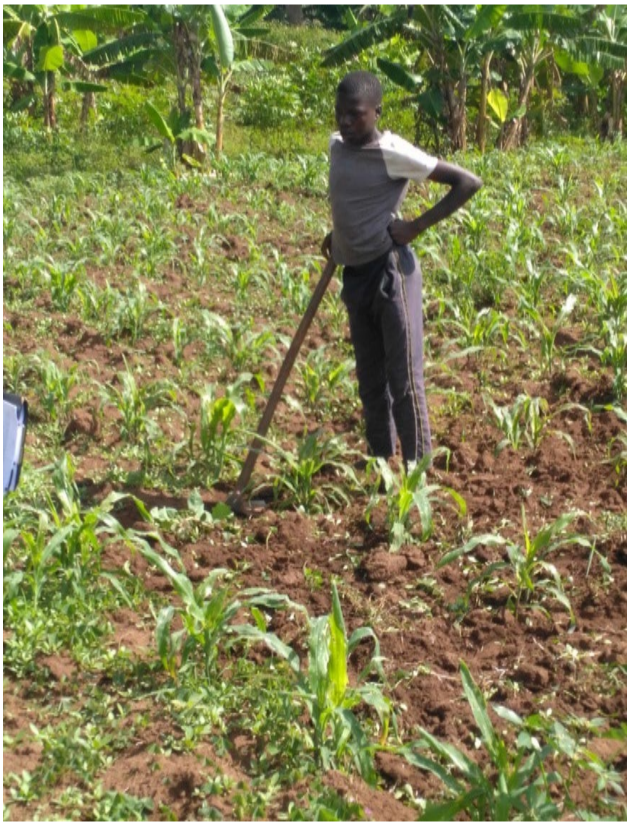

Fig. 4b: Weeding as one of the cultural methods practiced by farmers to keep maize fields healthy.

11.1.5 Results and discussion

Gender Profile of maize farmers

In Kamuli district, 24% of the farmers interviewed were female while 76% were male. In Namutumba, the majority (69.4%) were also male dominated farmers while females were 30.6% (Table 1).

Table 1. Maize farmer's profiles from Uganda's Kamuli and Namutumba districts.

Study variable	Kamuli	Namutumba
Gender profile of respondents (%)		
Female	12 (24%)	15 (30.6%)
Male	38 (76%)	34 (69.4%)
Educational level of farmers (%)		
Non-formal	1 (2%)	3 (6.1%)
Basic (elementary)	20 (40%)	20 (40.8%)
Secondary	25 (50%)	21 (42.9%)
Tertiary	4 (8%)	5 (10.2%)
Farmers experience in maize production		
< 10 years	3 (6%)	12 (24.5%)
10-30 years	32 (64%)	29 (59.2%)
31-50 years	14 (28%)	8 (16.3%)
51-70 years	1 (2%)	0
Membership to farmer organizations		
Yes	34 (68%)	27 (55.1%)
No	16 (32%)	22 (44.9%)
Source of planting materials		
Own seed	11 (22%)	13 (26.5%)
Government	5 (10%)	19 (38.8%)
Retail agro-shops	21 (42%)	15 (30.6%)
NGOs	13 (26%)	2 (4.1%)
Scale of production		
Small	24 (48%)	22 (44.9%)
Medium	25 (50%)	26 (53.1%)
Large	1 (2%)	1 (2%)
Type of cropping systems under maize		
Organic monoculture	0	0
Organic mixed cropping	0	0
Inorganic monoculture	27 (54%)	22 (44.9%)
Inorganic mixed cropping	23 (46%)	27 (55.1%)
Farmers' ability to identify FAW		
True	42 (84%)	43 (91.7%)
False	2 (4%)	0
No idea	2 (4%)	4 (8.2%)
Only symptoms	4 (8%)	2 (4.1%)

Educational profiles

An assessment of the maize farmers' education levels in the two surveyed districts showed the majority were educated to secondary level (i.e., 50% in Kamuli; 42.9% in Namutumba, see Table 1). Two percent of the farmers in Kamuli had undergone non-formal education compared to 6.1% in Namutumba. Additionally, 58% of the farmers in Kamuli and 10% in Namutumba had received tertiary level education (Table 1). Generally, a great majority of the maize famers in both districts were educated by Uganda National standards (UBOS 2006).

Farmer experience in maize production

Farmer's experience in maize production within Kamuli district ranged from 4 to 50 years while it was 1 year to 50 years in Namutumba. The majority of the farmers (i.e., 64% in Kamuli, 59.2% in Namutumba) reportedly had between 10-30 years' experience in growing maize (Table 1). 28% of farmers in Kamuli had between 31 and 50 years' experience with 16.3% of these in Namutumba district.

Scale of maize production

Very few farmers (2%) in the two districts were large-scale farmers growing more than 10 acres of maize. Majority of the farmers were either medium scale (50% in Kamuli, 53.1% in Namutumba) or small scale (48% in Kamuli, 44.9% in Namutumba) (Table 1).

Cropping system under maize

The most common cropping system amongst maize farmers in the two districts was inorganic monoculture maize (54% in Kamuli, 44.9% in Namutumba; Fig. 4c) or as inorganic mixed cropped maize (46% in Kamuli, 55.1% in Namutumba; Fig. 4d) (Table 1). Organic maize production was not found practiced anywhere by the farmers in the two districts.

Fig. 4c: Inorganic monoculture maize field in Kamuli district

Fig. 4d: Inorganic mixed cropping (maize with groundnuts and banana) in Kamuli district.

Source of maize seed used in planting

Farmers indicated they obtained seed for planting from four major sources: (i) own seed preserved from previous harvests, (ii) from supplies provided by the government; (iii) from non-government organizations, and (iv) purchased from retail agro-stockists. While 42% of farmers in Kamuli obtained seed from agro-stockists with 26% from NGOs, the farmers in Namutumba obtained their seed mainly from government (38.8%) and from retail agro-stockists (30.6%) (Table 1). Generally, many of the farmers in the two districts revolved seeds from previous harvests and this accounted for 22% of farmers in Kamuli and 26.5% of the farmers in Namutumba.

Membership to farmer organizations

Asked as to whether they belonged to any farmers' groups/organizations, 68% of the farmers in Kamuli and 55.1% in Namutumba responded in the affirmative (Table 1). Farmer groups or organizations were more structured and formal (legally recognised) in Kamuli than in Namutumba.

Total membership of these farmer organizations ranged from 8 to 4000 farmers in Kamuli although 54.8% of them comprised of 10-30 members. In Namutumba district, membership ranged from 20 to 349 members. However, 61.5% groupings comprised 20-30 members.

The Fall armyworm

The FAW has been recognised as a devastating pest since its invasion of the country and the African continent in general. Much appreciated is the fact that the FAW is much more devastating during the dry season than during the rainy season (NARO-ATAAS 2018). We sought to understand four key issues around the pest from the farmers' perspective: (i) year of first notice of invasion in farmers individual maize fields, (ii) the economic impact of the FAW pest in terms of yield loss by examining the yield before the official FAW invasion record and after FAW damage or symptoms were noticed, and included also the change in income before FAW and after FAW invasion, (iii) the farmers' current perception and appreciation of the status of the FAW from the time of first notice to date when they have applied some interventions to control or manage the FAW, and (iv) what management practices have been useful in controlling the FAW and if there have been any notice of failure in the management practices/interventions.

Year of FAW invasion

While the official report for FAW in Uganda was 2016, results of first notice of FAW symptoms and damage by farmers indicated the pest was noticed at different times depending on the locality within the districts. In Kamuli district, 2% of the farmers reported to have started to notice the FAW damage and symptoms in 2014, 22% in 2015, 10% in 2016, 40% in 2017, 20% in 2018 while about 2% noticed it in 2019 and 2020 in their localities (Fig. 5).

Within Namutumba district, 2% of farmers reported to have noticed the FAW as early as 2013 while others continued to notice it in subsequent years, 8.2% noticed FAW in 2015, 22.4% in 2016, 40.8% in 2017, 20.4 in 2018 while 2.1% noticed it in 2019. Generally, the majority of farmers interviewed in the two districts noticed FAW damage in the year 2017. 2% of farmers in Kamuli had no idea when FAW invasion occurred (Fig. 5).

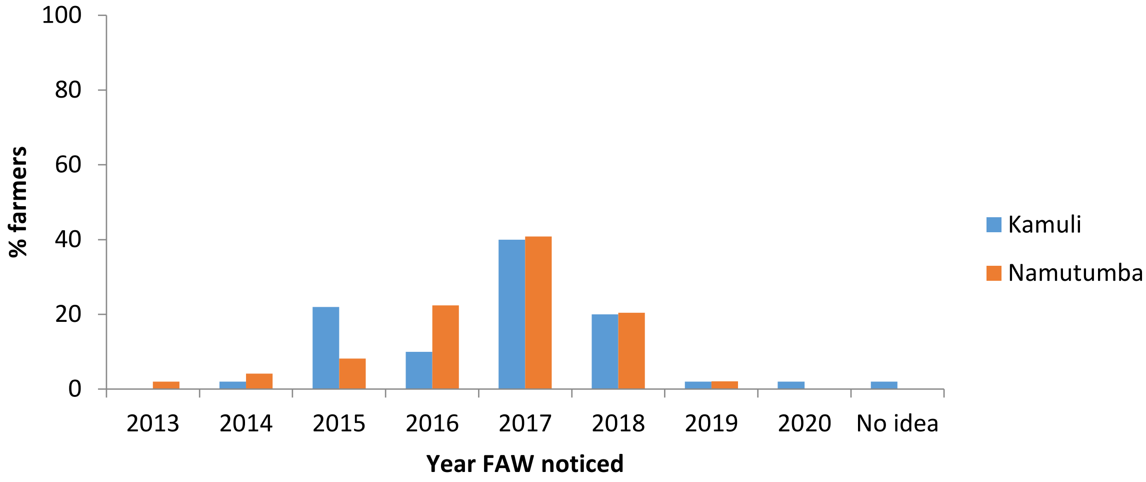

Fig. 5: Percentage of farmers that reported year they noticed first invasion of FAW in their fields.

Economic impact (Yield loss estimates)

The impact of the FAW in terms of yield loss reported by farmers was relatively variable. While 20% of the farmers reported yield losses less than 25% in Kamuli district, the numbers were much less at 10.2% in Namutumba district (Fig. 6). In Kamuli, 56% of the farmers experienced yield losses of 25-50% of the crop compared to 57.1% of the farmers in Namutumba who experienced the same magnitude of yield loss. 16% of farmers in Kamuli and 20.4% of farmers in Namutumba reported yield losses to the magnitude of 51-70%. A few farmers though (4% in Kamuli and 6.2% in Namutumba) experienced 90-100% yield loss due to FAW (Fig. 6). Generally, the highest percentage of farmers reported yield losses to be in the magnitude of 25-50% in both districts which consequently reduced their income as well.

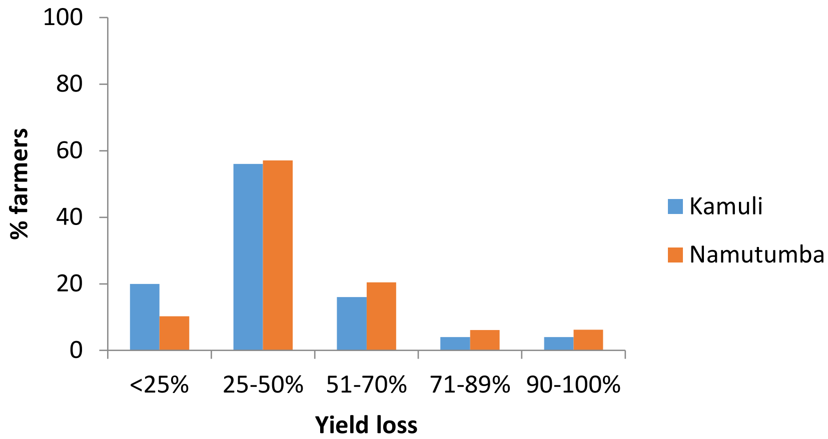

Fig. 6: Percentage of farmers that reported yield loss estimates under different categories in the two districts surveyed.

Asked whether FAW infestation reduced their income, 98% of the famers in Kamuli and 95.9% of those in Namutumba strongly agreed with the statement while 2% and 4.1% of farmers simply agreed with it in the two districts, respectively. 74% and 85.7% of the farmers in Kamuli and Namutumba, respectively, also strongly agreed that FAW was a threat to maize production in their respective areas/districts. Additionally, 92% of the farmers in Kamuli and 98% of those in Namutumba acknowledged the FAW reduced maize yield. Probed further to the effect that FAW reduced the cost of production, 82% of the farmers in Kamuli and 83.7% of the farmers in Namutumba strongly disagreed with it while 12% and 10.2% just disagreed (for Kamuli and Namutumba, respectively). About 2% in Namutumba district had no opinion about costs of production being lowered or increased by FAW infestation (Table 2).

Table 2: Kamuli and Namutumba Districts farmers' perceptions of economic impact of FAW.

Study variable	Kamuli	Namutumba
FAW infestation reduces farmers income		
Strongly disagree	0	0
Disagree	0	0
No opinion	0	0
Agree	1 (2%)	2 (4.1%)
Strongly agree	49 (98%)	47 (95.9%)
FAW is a threat to maize production		
Strongly disagree	0	0
Disagree	5 (10%)	1 (2.04%)
No opinion	0	1 (2.04%)
Agree	8 (16%)	5 (10.2%)
Strongly agree	37 (74%)	42 (85.7%)
FAW damage reduces maize yield		
Strongly disagree	0	0
Disagree	0	0
No opinion	0	0
Agree	4 (8%)	1 (2%)
Strongly agree	46 (92%)	48 (98%)
FAW reduces maize production costs		
Strongly disagree	41 (82%)	41 (83.7%)
Disagree	6 (12%)	5 (10.2%)
No opinion	0	1 (2.04%)
Agree	2 (4%)	2 (4.08%)
Strongly agree	1 (2%)	0

Farmers' perception of FAW

Of the farmers interviewed, the majority (64% in Kamuli, 81.6% in Namutumba) considered the FAW to still be very serious challenge to maize production in their localities (Fig. 7), while 22% and 16.3% of them thought it was just serious and could be controlled if they adopted the right approaches and intensified control operations.

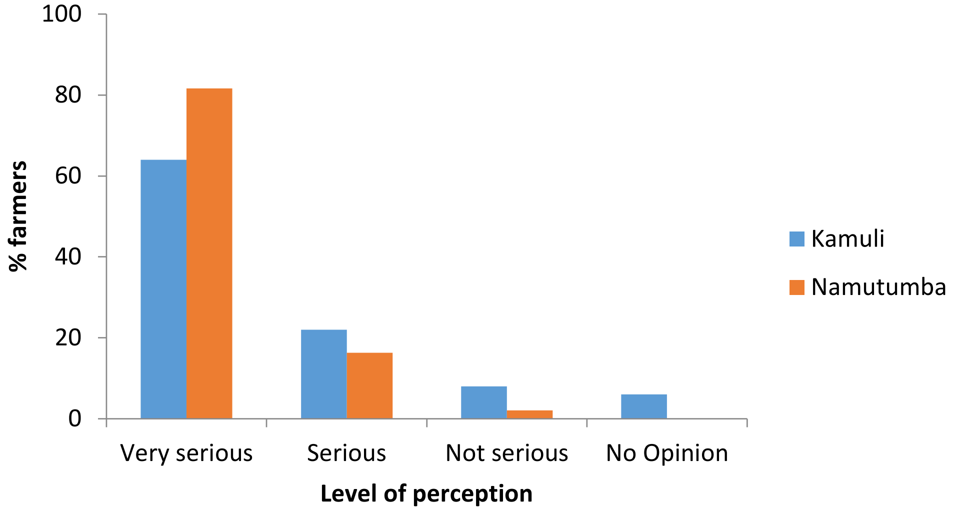

Fig. 7: Farmers levels of perception of the current FAW challenge in the two districts surveyed

Farmers ability at identification of FAW damage symptoms/larvae

An assessment of the level of knowledge to identify FAW in the field by description of its appearance and by the symptoms of damage revealed that 24% of the farmers in Kamuli and 87.8% of the farmers in Namutumba could correctly identify the FAW by its appearance as compared to the 8% and 4%, respectively, who could only identify FAW by symptoms so caused (Table 1). Farmers at first were asked to describe what they observed as perceived symptoms of FAW damage, and later were shown photos of various stages of the fall armyworm (life cycle) and symptoms, without telling them so they could identify what they had seen and observed in their fields.

About 4% of the farmers in Kamuli could not identify the FAW correctly, while 8% of the farmers in Kamuli and 4% of those in Namutumba had no idea as to either the appearance or the damage symptoms caused by FAW on maize.

FAW Farmer management practices

To manage the FAW, 84% of the respondents in Kamuli and 89.8% of those in Namutumba reported to use chemical insecticides with varying levels of successes and failures. Besides chemical insecticides, 42% of farmers in Kamuli and 44% of those in Namutumba managed FAW by the cultural practice of regular weeding (Fig. 4b). Some farmers (24% in Kamuli, 30.6% in Namutumba) tried physically and manually removing the FAW larvae (hand picking) from infested maize stands and cobs. However, the method was very laborious and difficult to sustain especially those for farmers with relatively sizeable fields beyond one acre. About 4% of farmers in both Kamuli and Namutumba reportedly took no action against FAW citing various reasons such as poverty and cost of insecticides. Some of those who took no action reported they were simply overwhelmed by the devastating effect of the FAW and abandoned the maize fields since they could not access or afford effective control measures.

The use of pheromones and biological control as methods to manage FAW was not reported by farmers from the surveyed districts, however the use of biological extracts (Pepper, Tobacco, *Aloe-vera*, *Lantana*, Sisal) was evident although not common and therefore not frequently used by farmers (details in Appendix III).

Table 3: List of insecticides commonly used against FAW by farmers in the two districts surveyed.

Trade name	Active ingredient (a.i)	WHO class*	Recommended dosages	
			In 15L	In 20L
Rocket	Profenofos Cypermethrin	II	15-40	20-50
Amdocs	Emamectin Abamectin	II	25-30	30-50
Profecron	Profenofos Cypermethrin	II	15-40	20-50
Striker	Lambda cyhalothrin	III	15-20	20-25
Tafgor	Dimethoate	II		
Eminent	Emamectin benzoate	IV	4 tea spoon (6g/tea spoon)- 6-9mls	5 tea spoons 8-12 mls
Dudu acelamectin	Abamectin	II		
Duducyper	Cypermethrin	II		
Laraforce	Lambda cyhalothrin	III		

*WHO classification: II = moderately hazardous; III = slightly hazardous; U = unlikely to present acute hazard in normal use

The majority of farmers in the two districts used Rocket (a.i.: profenofos/Cypermethrin) as their main insecticide against the FAW (44% in Kamuli, 42.8% in Namutumba). The second most commonly used insecticide in Kamuli was Striker (a.i.: lambda-cyhalothrin) and was used by 20% of the farmers while in Namutumba, Eminent (a.i.: Emamectin benzoate) represented the most widely used insecticide (i.e., 16.3% of the farmers) followed by Duducyper (a.i.: Cyptermethrin, 12.2%) and Striker (a.i.: Lambda cyhalothrin, 10.2%), respectively. World Health Organization (WHO) classifies Profenofos/Cypermethrin as class II (moderately hazardous) pesticides, lambda Cyhalothrin as class III (slightly hazardous) pesticides, while Emamectin benzoate is class IV (unlikely to cause acute effects in normal use) pesticide.

Regarding the method of application, most of the farmers in the two districts (84% in Kamuli, 87.8% in Namutumba; Table 4) used targeted spraying in the maize funnel as opposed to random spraying so as to directly target the funnel where the caterpillars resided. 80% of the farmers in Kamuli and 87.7% of those in Namutumba applied only one chemical pesticide at a time within the cropping season, while 8% and 4.1% attempted to spray more than once within the season in the two districts, respectively. Most farmers attempted to spray either two or three times, usually after two weeks (Table 4). At least 60% of the farmers interviewed had spraying equipment. However, the majority (46% in Kamuli and 75.5% in Namutumba) did not have protective gear. Of those that reported to have protective gear, their use was very low, for example, 50% of the farmers in Kamuli and 87.8% of those in Namutumba did not use them while spraying the chemicals. A few farmers (28% in Kamuli, 28.6% in Namutumba) reported to use protective foot ware (i.e., gumboots) and also handkerchiefs as improvised face masks. There was no use of gloves and work suits reported. Even when farmers were aware of potential side effects on their health (e.g., skin irritation and headache) as consequences of not using protective gear, farmers simply reported that it was expensive for them.

Table 4: Parameters regarding method of chemical application and frequency of use

Study variable	Kamuli	Namutumba
Chemical use within season		
One chemical at a time	40 (80%)	43 (87.7%)
Two or more at once	4 (8%)	2 (4.1%)

Frequency of use		
Once	9 (18%)	4 (8.2%)
Twice	13 (26%)	16 (32.7%)
Thrice	18 (36%)	16 (32.7%)
Four times	1 (2%)	2 (4.1%)
No schedule	2 (4%)	1 (2%)
Spraying equipment		
Yes	31(62%)	30 (61.2%)
No	13 (26%)	16 (32.7%)
Protective gear		
Yes	22 (44%)	9 (18.4%)
No	23 (46%)	37 (75.5%)
Protective gear use		
Yes	20 (40%)	3 (6.1%)
No	25 (50%)	43 (87.8%)
Method of spraying		
Random spraying	1 (2%)	0
Targeted spraying	42 (84%)	43 (87.8%)
Noticed chemicals failing[1]		
Yes	28 (56%)	28 (57.1%)
No	17 (34%)	15 (30.6%)
No idea	5 (10%)	4 (8.2%)

[1]By chemical failing, the farmers meant that the FAW could survive and thrive after application of the chemical, i.e., chemical was ineffective to control FAW

Besides Chemical control of FAW, the other methods used by farmers were majorly cultural methods and also the use of biological extracts. Cultural methods of FAW control used included adequate land preparation, garden sanitation, crop rotation, intercropping, hand picking, early planting, use of organic manure to enhance crop growth, and a habitat management practice commonly known as push-pull strategy. Originally developed for the control of cereal stem borers, push-pull strategy involves intercropping maize (or another cereal crop) with a legume crop (e.g., desmodium) and this is simultaneously intercropped with nappier grass at the periphery (edges) of the garden. The desmodium intercrop ideally repels or 'pushes' pests away from the maize while the nappier grass 'pulls' the pests away from the maize. A farmer in Kamuli reported that the method was 100% effective in controlling FAW in his field while another reported 70% effectiveness.

Biological-based methods for FAW control involved the use of animal and plant products. The animal product was majorly urine (animal/human urine) while plant extracts were mainly from *Aloe vera,* Tobacco (*Nicotiana tabacum*), Chili pepper (*Capsicum* sp.), *Lantana camara* and the Neem tree (*Azadirachta indica*) used alternately or in combination with Ash. A detailed description of each of these methods is available in Appendix II.

Farmer's sources of FAW information

An assessment of how farmers accessed and/or shared information on the FAW revealed two main sources: (i) farmer to farmer exchanges/farmers groups, and (ii) TV/Radio programs/talk shows (Fig. 8). In Kamuli, farmers relied mostly on TV and radio programs (38.6%), followed by farmer-farmer exchanges either individually or in farmer groups (32.9%), while others depended on the government extension service available in the district (21.4%). 7.1% of farmers interviewed relied on indigenous knowledge and personal experiences. Within Namutumba district, farmer-farmer exchanges formed the main source of information (36%), followed by TV/Radio programs/talk shows (28.6%), government extension service (22.9%), indigenous knowledge/own experience (12%), and agro-stockists (4%).

While farmers used all channels to receive information on FAW, much of the sharing of agricultural information in general was through exchanges between farmers either individually or in groups. A number of non-government organizations allied to agriculture in the two districts have organized farmers in groups through which they could share a wide range of information on agronomic practices including pest and disease management, and post-harvest and marketing. In the two districts, none of the people interviewed had ever heard or even used the FAW app FAMEWS developed by the FAO.

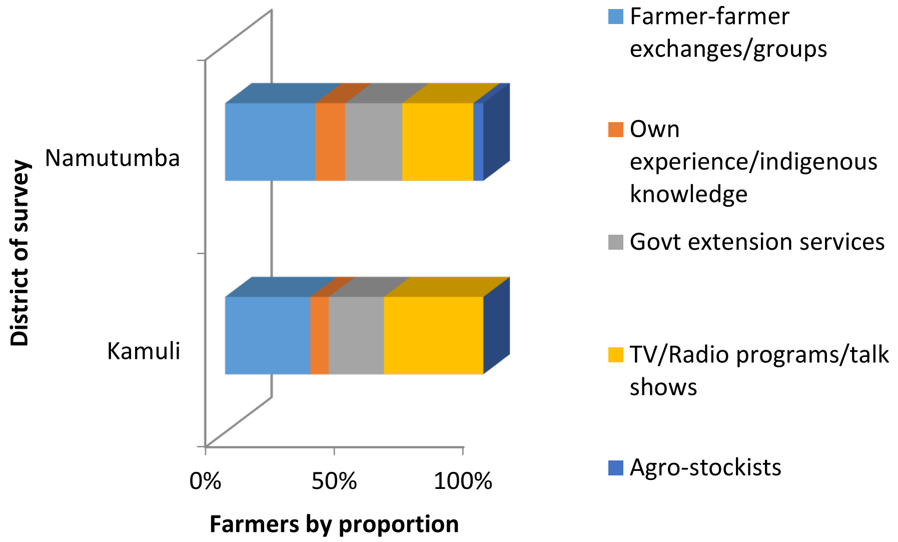

Fig. 8: Sources of farmer information on FAW information in the two Ugandan districts surveyed.

11.1.6 General discussion

This study aimed at documenting practices that have been useful in controlling the fall armyworm since its invasion of Africa using Uganda as a case study. Farmers' practices, the actual actions employed to combat the FAW, the perceptions of farmers which help to understand and also predict whether pest control efforts can be successful given the diversity in community behaviours were documented. All these are good tools for designing sustainable pest management strategies for this pest.

After its invasion, many African Governments provided and distributed pesticides for use by the farmers who have continued to use this approach as the first line of control measure against the FAW. The farmers have provided more information on the type of chemical insecticides used, dosages, spray regimes, and how they handled the pesticides although there was little regard towards safety. Farmers also try to use inflated dosages of some pesticides while others combined them in a desperate attempt to control the FAW with the belief that the effects on the FAW would be overwhelming but instead noticed failure. The 56% of farmers in Kamuli and 57.1% of farmers who noticed chemical use failing could be attributed to some of the above reasons. Use of inflated dosages could lead to the development of resistance either singularly or in combination with other insecticides. The method of application of these chemicals and the frequency of use may influence efficacy. Pesticide use therefore needs to be re-evaluated and urgent research (dosages, when and how they are applied) instituted as their frequent use may have serious implications on environment, human and animal health.

Through this study, we were also able to assess farmers' knowledge of the FAW (i.e., what they knew about it and their ability to identify the pest's different life stages), and also understand their perceptions about pest status, damage, and the effectiveness of control measures at their disposal. Farmers were able to rate the severity of the FAW and while the yield estimates provided a measure of quantification of crop damage, the accuracy of such estimates remained to be ascertained. The information represents farmer's perception of the damage inflicted on the crop by this invasive pest (Schreinemachers et al. 2015).

Besides chemical pesticides, some cultural practices such as frequent weeding, intercropping and trap cropping were used by smallholder farmers to manage the FAW. The push-pull method, which involves intercropping maize with some leguminous crop and has been shown to be effective against various cereal stemborers when compared to monoculture maize (Midega et al. 2018), was noted as effective against the FAW by some farmers from Kamuli. The push-pull systems have been found to increase plant diversity and could encourage and conserve natural enemies present in the agricultural landscape (Day et al. 2017). Other non-chemical practices such as the use of ash, urine, sand, soil, and plant extracts (such as *Capsicum* sp., *Lantana camara*, *Azadirachta indica*, *Aloe vera*, *Agava sisalana*) represented cheaper options for poor farmers (Stokstad 2017; Kumela et al. 2018) and have shown conflicting potentials for the management of the FAW, with some farmers noting these approaches as being labour intensive. Phambala et al. (2020) found potential bioactivity of some of these extracts against the FAW with high mortality (about 50%) reported for *A. indica* and *N. tabacum* and < 40% recorded for *Aloe vera*. In Ethiopia, Sisay et al. (2019) also reported high activity of some of these extracts including *L. camara*, *A. indica, N. tabacum* and *Jantropha gossypifolia* against the FAW. In Brazil, Silva et al. (2015) found aqueous extracts of neem seed cake to be effective against the FAW in maize. Cultural approaches and options involving biological extracts typically have low associated health and environmental risks (Prasanna et al. 2018). These and many other practices that show potential (given the perceptions of the farmers) require scientific validation before they could be promoted for use and adoption by the wider farming community across the recent invasive ranges of the FAW (e.g., Near East, Asia, Southeast Asia, Pacific Island nations).

Although many farmers strongly agree that the FAW is still a challenge to maize production, the majority now are confident that they now have several management and control options to reduce the damage posed by the FAW, and with adequate public education and sensitization, they believe the FAW will be contained through area-wide approaches. It is because of this that some farmers strongly disagreed with the statement that FAW is a threat to maize production reasoning that they only thought so in the beginning when they had no known control options.

In the two districts surveyed, farmers were not aware of the existence of the FAW app as a source of information, and very few farmers (less than 1%) had smart phones or internet access. Perhaps the use of such tools (FAW app) and access to internet could help strengthen control as farmers could be able to find more relevant and updated information on FAW management practices. Although officially reported in 2016, some farmers reported they noticed the FAW symptoms and damage as early as 2013 in Namutumba, some in 2014 in Kamuli but some had at first confused it with the cereal stemborers in which case the FAW was more devastating than these. Given the severity of FAW damage, it became evident that this was a new and different invasive species. Some farmers could recall the exact period when they first noticed such devastating damage and symptoms given their current level of awareness, experience, and ability at identification (though still low in some areas). They were therefore confident that they noticed FAW earlier than officially reported.

Given the farmers' educational level, the farmers believe they have the ability to manage the FAW if they have the right and efficacious chemical insecticides as they are able to correctly apply them and follow recommended procedures. The majority therefore perceive that soon they will be able to achieve total control of this pest.

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11.1.7 Appendices

Appendix I. Sample questionnaire used for data collection

SEA FAW PROJECT- DOCUMENTATION OF PRACTICES THAT HAVE BEEN USEFUL IN AFRICA/SOUTH EAST ASIA FOR MANAGING FAW BY FARMERS- The case of Uganda (Interviews with farmers/discussions with regional extension staff/champion farmers to gather knowledge not included in scientific literature).

District--
Location (Village) ---
GPS ---
Date of Interview---
Time of Interview---

1. FARMERS DETAILS

(a) Name of Farmer--Tel. contact----------------------
(b) Gender (sex) ---Age----------------------------------
(c) Educational level………………. (e.g., Non-formal, basic, secondary, tertiary)
(d) Are you a member of any farmer-based organization?..........................
If yes, number of members……. ……………………
(e) Permission to disclose personal information, Yes---------------------No------------------------

2. FARMERS ECONOMIC PROFILE (based on maize)

(a) Size of household………………………………………………………………
(b) Yields per ha/acre (before FAW arrived)………………………………………
(c) Income per ha/acre (before FAW arrived)………………………………………
(d) Yield loss due to pests (FAW)…… (on scale <25%, 25-50%, 50-70%, 71-80%, 90-100%)

3. MAIZE PRODUCTION PROFILE

(a) Age of maize plants grown………………………………………………
(b) Varieties of maize planted………………………………………………
(c) Source of maize for planting………(e.g., Government, NAADS, Businessmen/women, Middlemen/women, etc)
(d) Number of years in maize production………………………………………………
(f) Scale of production-…………..(e.g. small, medium or large (i.e. for plantation crops, large= >10 ha, medium = 2-9 ha, small = < 2 ha))
(e) Type of cropping system………(organic mono crop, organic mixed crop, inorganic mono crop, inorganic mixed crop).

4. FARMERS AWARENESS OF FAW AND PEST IDENTIFICATION

(a) Have you ever seen/heard of FAW? Yes------------------/No----------------------------------
(b) If yes, where did you see/hear about it? (own experience, fellow farmers/organization, maize traders, agriculture extension agents, researchers, radio/tv, others (friends and relatives, brochures/manuals etc.).
(c) When was the first time you saw/heard of FAW (or saw symptoms of it)?--------------------
(d) What is your perception of FAW in this area?--------------------(very serious, serious, not serious, no opinion)

5. FARMERS KNOWLEDGE OF FAW DAMAGE AND ECONOMIC IMPACT

Rank your knowledge with regard to FAW with the following responses;(1-strongly disagree 2-disagree 3-no opinion 4-agree 5-strongly agree)

No.	Characteristic knowledge	Response
1	FAW infestation reduces farmers income	
2	FAW are a threat to the Maize production	
3	FAW damage reduces maize yield	
4	FAW reduces production cost	
5	FAW is a pest of quarantine problem	
6	FAW eggs are laid inside the kernel	
7	Adult FAW do not feed on the Maize plant	

6. ABILITY TO IDENTIFY FAW PESTS

Farmers who show awareness of FAW problem are further tested for their ability to identify the true FAW pests (colour photographs/specimens of these insects are provided without names)
True identity-------------------------------------False identity--

7. FAW MANAGEMENT PRACTICES

(a) What efforts do you use to control FAW pests encountered on your farm? E.g., see in table.

No.	Intervention	Used/ not used and reason for use/not use
1	Pheromone trapping	
2	Spraying with chemicals	
3	Removal of FAW infested cobs	
4	Regular weeding and prompt harvesting	
5	No action	
6	Other practices you have used? Mention them	

Have you noticed chemicals or other control methods failing? Yes-----------------No------------

(b) If you use chemicals, what are their names…………………….or does not know name?

(c) How do you use chemicals within the season? ……… (One chemical at a time, 2 or more at once?)

(d) How frequently do you use a chemical (s)?............ (weekly, twice a week, once a month, fortnightly, no schedule).

(e) Do you have spraying equipment? Yes-----------------------------No------------------------

(f) Do you have protective gear? Yes----------No--------- and if you have, do you use them when spraying? Yes-------------------No-------------------

(g) How is spraying done? (Random spraying, targeted spraying?)

(h) What dosage do you use? (as recommended by manufacturer or devised by you-Lower---------------or higher------------------than recommended?)

(i) Which recommended FAW management practices do you know? check below table;

Other than or before chemical use, what other methods have you used previously to manage FAW? Was it effective (compare- cost, frequency of use, residues, higher price premium for maize)?

No.	method	Comments
1	Biological control	
2	Bait application	
3	Soil inoculation with bio-pesticides	
5	Others, mention them	

8. WAY FORWARD FOR MANAGEMENT OF FAW PESTS

(a) What in your opinion is needed to manage the FAW problem in your area? Rank them by priority; Some examples;

No.	Farmers suggestions	Some examples!
1		Strengthening agriculture extension
2		Farmer training
3		Public education
4		Improved research

5		In put subsidy/availability of recommended inputs (chemicals/equipment) for controlling FAW pests
6		Logistical support
7		Others?
8		No idea

(b) What ways (channels) have been useful in getting FAW information?

(c) Are you aware of the availability of any FAW apps?

(d) How can we enhance sharing of pest/agricultural information for (a) women and (b) men farmers information?

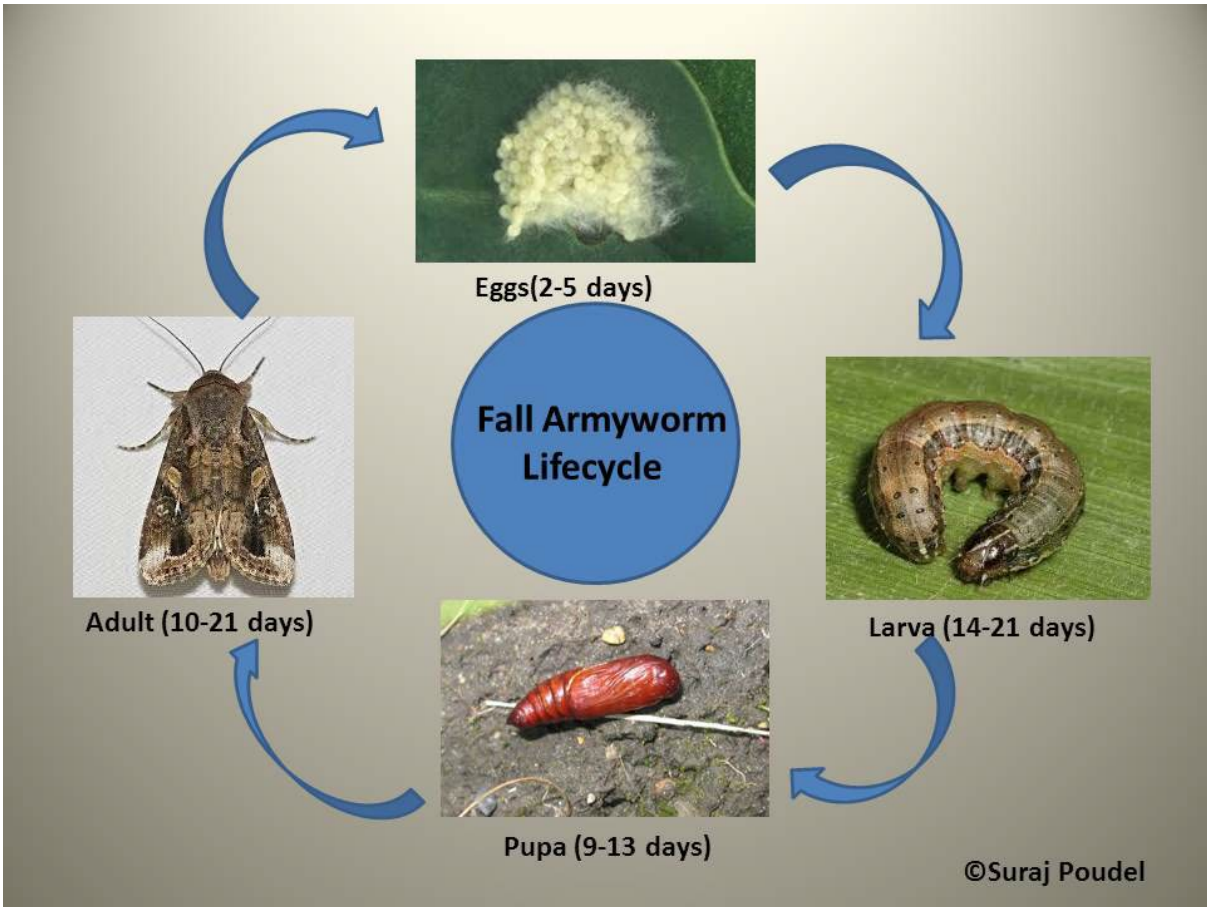
No.	Women	Men
1		
2		
3		
4		
5		
6		
7		
8		

END

Thank you for your time

AK

Appendix II. Pictorial chart of the FAW life cycle



Appendix III: A detailed description of farmers' FAW cultural and biological-based management practices

- Ash - A farmer in Kamuli reported use of ash prayed in the maize funnel and reported that ash kills young larvae but later stages of the caterpillar may not die with ash. Method is conducive for small scale gardens as it is laborious. Other farmers however reported it was not effective in their garden. A farmer in Namutumba reported to have used dry ash with no effect.
- Hand picking and killing the larvae was attempted in a small field and was effective but laborious. One farmer handpicked and bottled the larvae to kill them.
- Using petrol mixed with paraffin - A farmer in Kamuli reported use of this and it gives limited success against the FAW larvae.
- Flooding plant with water inside maize funnel - A farmer reported to run adequate amounts of water down the maize funnel where the FAW larvae were resident as having reduced the FAW but this was laborious and not sustainable.
- Manually squeezing/killing the FAW with sticks inside the funnel was practised by a farmer in Kamuli.
- Waiting for rainy season - Some farmers reported to be overwhelmed by the FAW during the dry season as it becomes very devastating during this period. To overcome serious losses, they have smaller areas under maize during dry season and increase acreage during the rainy season as the effect is minimal at the time.
- Ash + salt mixture in water- A farmer in Kamuli used this and reported some reduction in FAW incidence and damage. This needs further verification.
- Paraffin + Rocket + salt mixed in water – A farmer in Kamuli reported using 30 ml of paraffin mixed with 30 ml of Rocket (profenofos + cypermethrin) and 2 spoons of salt mixed in 20 litres of water and sprayed on the maize plants to give some considerable protection/control against FAW.
- Ash + Chili pepper –A farmer in Kamuli reported to use half kilogram of ash with half litre of Chili pepper solution mixed in 20L of water to control FAW when sprayed in maize funnel. Another farmer reported it had slight effect on the FAW. Another farmer categorically stated the mixture was 30% effective against the FAW larvae in maize.
- Chili pepper + human urine + ash – A farmer in Kamuli reported to use this combination (1/2 kg ash + 1 litre of urine + ½ kg Chili pepper) which after being mixed was left for five days to ferment and when decanted and sprayed inside maize funnel was 60% effective. Another farmer also in Kamuli used 2 litres of urine in half litre quantity of Chili pepper and ash which were fermented for 3 days, and without further addition of water used the mixture directly to spray into the maize funnel with very successful results against the FAW. A third farmer using the mix (2 litre urine, ¼ kg Chili pepper, ¼ kg ash) mixed with paraffin in a 20L knapsack sprayer reported 50% effective against the FAW. A fourth farmer used the above combination of urine, ash and Chili pepper in 3 L water which was decanted and sprayed and reported also about 50% effectiveness.
- Chili pepper + ash + salt – A farmer in Kamuli reported to use this mixture when left overnight. At first, it gave some control of FAW when sprayed on the plant but later it was not effective. Another farmer who used 2 half litre cups of Chili pepper with 3 half litre cups of ash and 2 spoons of salt in 10 litres of water reported a reduction in the damage due to FAW but found it did not kill the FAW in the long run.
- Adequate land preparation before planting - Some farmers reported that by practising adequate land preparation measures i.e., undertaking a 2nd and 3rd cultivation after some time interval after the primary cultivation, and before planting, the damage by the FAW was drastically reduced.
- Crop rotation - Some farmers reported that alternating maize crop with other crops (such as beans, soybean) helped reduce the incidence of infestation by the FAW.
- Human urine + tobacco + chili pepper – A farmer in Kamuli reported to use this combination and left it to ferment for 1 week and without addition of water sprayed in maize funnel killed 80% of FAW. This farmer reported the concoction to kill faster than the chemical insecticide Rocket.
- Garden sanitation - Some farmers (3 in Kamuli district) reported to use good hygiene practices such as the burying of maize residues after harvest or infested ones as a measure that helped to reduce FAW infestation in their gardens.
- Herbicide use instead of weeding - A farmer (in Kamuli district) reported to the use of herbicides to kill the weeds instead of weeding by hand-hoes as having helped to reduce on the incidence of FAW.
- Early planting - A farmers in Kamuli district realized that maize planted early on in the season (about a month) tended to be less affected than that planted late in the season and are using this to lessen damage by the FAW.

- Some farmers (2 in Kamuli district) targeted maize growing only when there were heavy rains and reported this to provide some degrees of success in control
- Intercropping as control strategy - A farmer in Bukuluba, Namutumba district reported that by planting more than one crop in a field, they were able to reduce the incidence of the FAW. Though the practice is for reasons such as the limited availability of land, the practice has helped them reduce the damage due to FAW. Crops commonly planted along with maize included beans, groundnuts and soybeans.
- Push-pull strategy - A few farmers reported to have been trained to use push-pull system by the International Centre of Insect Physiology and Ecology (ICIPE), Kenya. Originally developed for the control of cereal stem borers, the practice involves intercropping maize (or another cereal crop) with a legume crop (e.g., desmodium) and this is simultaneously intercropped with nappier grass at the periphery (edges) of the garden. The desmodium intercrop ideally repels or 'pushes' pests away from the maize while the nappier grass 'pulls' the pests away from the maize. In Kamuli, one farmer reported that the method was 100% effective in controlling FAW in his field while another reported 70% effectiveness. The few farmers (4%) that used it reported it as being 75-100% effective at controlling the FAW. The method is also used to control striga, a weed that is common in cereal cropping systems and hinders crop growth. Farmers reported the push-pull system is still under demonstration to the farmers but may be widely adopted by the farmers except that the seeds of desmodium and nappier grass are difficult and expensive to procure.
- Using fertilizers – Some farmers reported to use fertilizers to speed up plant growth before FAW attack and to overwhelm it. A few farmers reportedly used organic manure (cow-dung, chicken manure) while others used DAP (Diammonium phosphate) and Supergro provided to them by non-government organizations under their farmers groups. Some farmers (2 in Namutumba, 1 in Kamuli) that applied some fertilizers indicated that the fields where they applied were less affected when compared to those without any fertilizers.
- Use of ash sprayed on the leaves and inside the maize funnel was reported to reduce damage by the FAW as it reduced the feeding activity of FAW
- *Aloe vera* - A farmer in Nawampiti, Namutumba district reported to use *Aloe vera* extract which was boiled, left to ferment for 1 week and sprayed using knapsack sprayer in the maize funnels, and this was very effective against FAW.
- Tobacco + Chili pepper – The same farmer in Nawampiti, Namutumba district alternatively reported to use Tobacco (*Nicotiana tabacum*) + Chili pepper (*Capsicum* sp.) combination fermented for two days and when sprayed was effective against FAW larvae.
- Animal (cow and human) urine + Tobacco (*Nicotiana tabacum*) + *Lantana camara* extract + Neem extract (*Azadirachta indica*) + ash – A farmer in Namutumba reported to have used this combination, fermented for 2 weeks and sprayed on maize inside the funnel and it was effective against FAW larvae.
- Human urine + ash + Sisal (*Agave sisalana*) fluid extract - A farmer in Namutumba district reported to use half litre of urine with 1/2 kg of ash mixed in sisal fluid (liquid of comparable quantity) all mixed with 1 L of water and fermented for 2 weeks and when sprayed in maize funnel, the concoction was 95% effective against the FAW. However, the farmer reported the practice was labour-intensive.
- Using OMO detergent - A farmer in Namutumba reportedly used the detergent 'OMO' and it was effective against the FAW. However, another farmer reported to have used without any effect on FAW larvae.
- Ash + urine + OMO detergent + paraffin – A farmer in Buwaga, Namutumba district reported to use a combination of these (1/2 kg ash, 2 L urine with OMO detergent and an addition of paraffin) fermented for one week and this was reported to be effective against the FAW.
- Intensive weeding of about 7 times - some farmers reported that by weeding about seven times (or as frequently as possible), they could reduce FAW damage on their maize crop. This potentially could lead to continuous exposure of pupal stages (usually under the soil) to extreme temperatures (e.g., heat) on the surface or increase their chance of being predated.
- Sand - A farmer in Namato, Namutumba district reported to use sand inside the growing points of maize (funnel) and reported that this killed the FAW larvae but it was a laborious practice used for small gardens.

11.2 Appendix 2: CARDI Report in lieu of Insecticide Bioassays

Updates on *Spodoptera frugiperda* (fall armyworm, FAW) in Cambodia

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Reporting date: 31st March, 2022

Key Findings

- Limited understanding of the new exotic lepidopteran pest Spodoptera frugiperda for both scientific researchers and farmers in Cambodia
- Pest infestation levels and severity to maize crop damage differed between different districts and provinces
- There is currently a lack of national coordination with respect to usage of chemical insecticides for the management of S. frugiperda in Cambodia
- There is a desire by CARDI researchers to better understand this genetic characteristics of the FAW populations in Cambodia
- Challenging local legislations relating to sharing of biological material, including sharing of non-native invasive species with international collaborators, have thwarted this effort which could significantly and negatively impact local farmers' ability to manage the pest and their livelihood

Future opportunities

- Better research on IPM options for the management of Spodoptera frugiperda in Cambodia
- International collaborations to understand the population genomics of *S. frugiperda* at national and regional scales
- Research opportunities to explore cultural pest management strategies that local farmers could adopt
- Surveys to understand the prevalence of the pest across different agricultural systems in Cambodia

11.2.1 Introduction

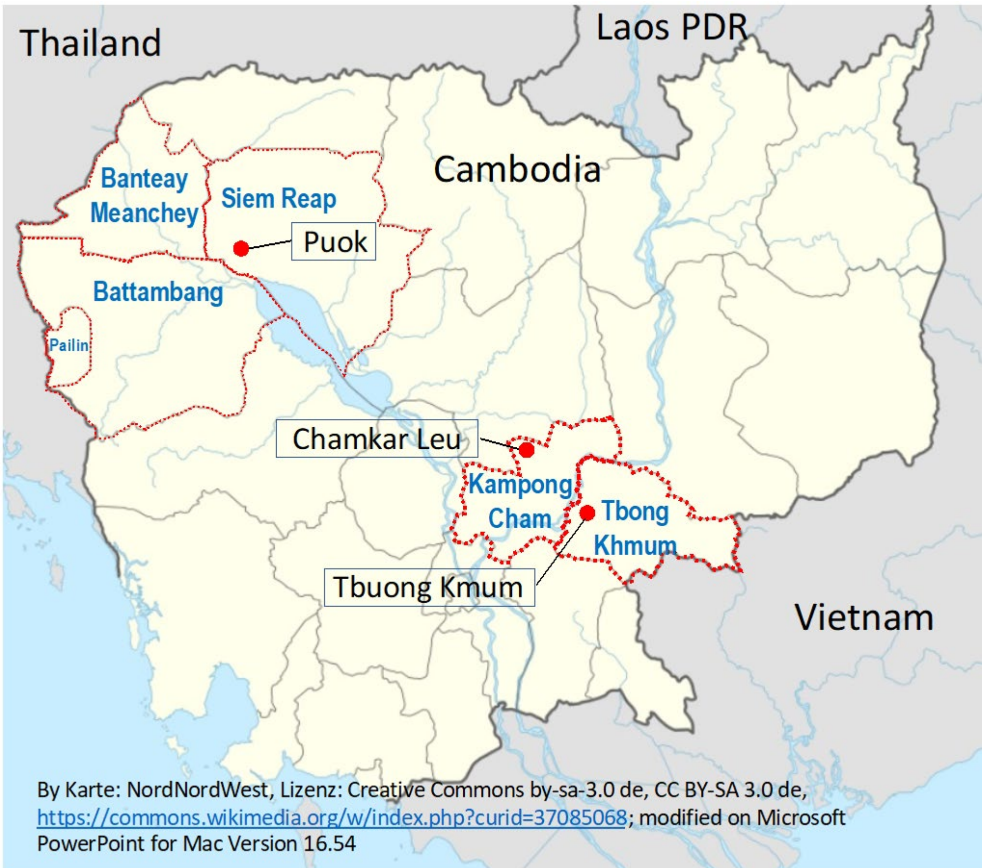
The maize in Cambodia has been heavily damaged by a new exotic species of lepidopteran pest widely known as the fall armyworm (FAW) *Spodoptera frugiperda*. This pest is native to the Americas and thrives in sub-tropic/tropical climate conditions, with population range extending from southern North America to Central America and into South America. Although first confirmed to be present in western Africa in 2016 (Goergen et al. 2016), it has also been reported in Southeast Asia (SEA) and Asia since 2008 in Vietnam (Vu 2008; Nguyen and Vu 2009) and in south China since 2016 (Tay and Gordon 2019). The presence of *S. frugiperda* in Cambodia from damaged maize fields was officially reported for the first time in May 2019. The genetic diversity and insecticide resistance profiles of *S. frugiperda* populations in Cambodia are currently unknown. Its proposed population origin is assumed to be from western Africa as a result of a founder event (e.g., Goergen et al. 2016; Nagoshi et al. 2017; Day et al. 2019) and from the rapid spread of the pest due to its strong flight ability (Rose et al. 1975; Jones et al. 2019) although spread involving human-assisted international trade and via tourism activities have also been proposed (Early et al. 2019).

Recently, population genomics studies involving African and Asian FAW populations (Tay et al. 2022a; Schlum et al. 2021), SEA, East Asia (e.g., South Korea), Pacific (e.g., PNG) and Oceania (e.g., Australia) FAW populations have shown that multiple independent introductions of S. frugiperda underpinned the perceived rapid spread of this pest and is contrary to the widely accepted axiom of a western African origin (Rane et al. 2022a). Instead, gene flow patterns identified Asia and SEA regions as biosecurity hotspots that also played crucial roles in the spread of the pest, including multiple introductions of the pest to Asia (Jiang et al. 2022; Tay et al. 2022a), to SEA (Rane et al. 2022a), and likely an east-to-west spread that linked SEA populations with east African FAW populations (Tay et al. 2022a, Rane et al. 2022a). Bioassay experiments and genome-wide resistance allele characterisation of invasive FAW populations (e.g., Tay et al. 2022b; Eriksson 2019; Lv et al. 2021) also did not support the African origin of FAW (Tay et al. 2023). There is currently no genomic information for the *S. frugiperda* in Cambodia.

Spodoptera frugiperda were found in four provinces (Pailin, Battambang, Banteay Meanchey, and Tbong Khmum; Fig. 1), and affected a total 11,142 ha of maize crop. The Minister of Agriculture, Forestry and Fisheries, H.E. Veng Sakhon, reported in June 2019 that as of June 11, a total of 11,142 hectares of corn fields were destroyed which consisted of 2,544 hectares in Pailin; 3,033 hectares in Battambang; 4,715 hectares in Banteay Meanchey and another 850 hectares in Tbong Khmum province. In order to estimate the level of damage caused by the FAW on farmers' corn fields, the Plant Protection Division of the Cambodian Agricultural Research and Development Institute (CARDI) also started assessment in the first week of August, 2019.

The aims of this report are to provide: (i) a documentation of the detection of *S. frugiperda* in Cambodia; (ii) an initial assessment of severity and incidences of crop damage by the FAW in selected provinces and districts in Cambodia; (iii) report the outcomes of initial assessment of management options using two biopesticides (Neem Oil, entopathogenic fungus *Beauveria bassiana*); and (iv) identify future research opportunities needed to assist farmers to manage this invasive pest.

Fig. 1: A map showing the districts affected by *Spodoptera frugiperda* and the district where maize crops were assessed by CARDI. *S. frugiperda* was first confirmed from four provinces of Pailin, Battambang, Banteay Meanchey, and Tbong Khmum in May 2019. Assessments of maize crop damages by *S. frugiperda* from the districts of Puok, Chamkar Leu and Tbuong Kmum from the Provinces of Siem Reap, Kampong Cham, and Tbong Khmum, respectively, are also shown.



11.2.2 Methods

Identification of *Spodoptera frugiperda* (FAW)

Larvae were identified as FAW based on morphological features (i.e., the 'inverted Y' and four black dots that appeared on the second final segment of the body) although their strain identity (i.e., C- or R- strains) were not determined. At the time of field surveys in August 2019 as undertaken by CARDI, most of corns were in maturity stage, and three districts (i.e., Chamka Leu, Tbong Khmum, Pouk) from Kampong Cham, Tbong Khmum, and Siem Reap Provinces, respectively, were selected for evaluation.

Crop damage evaluation

Crop damage evaluation involved estimating both incidence and severity (% calculated, Table 1) of infestation and was carried out by Dr. Khay Sathya and Plant Protection Division of CARDI. Methods of estimating incidences and severity of FAW damage are as outline below:

Incidence:

Step 1. Randomly count 100 corns from each farm for three different places (i.e., three replications), and check for the presence or absence of FAW.

Step 2: Detection of FAW on each maize plant is counted as 1, while absence of FAW on a plant is counted as zero (e.g., Rep-I (100 corns) = 92% (represents 92 corns' ears being infested by FAW out of 100 corn counted).

Severity:

Step 1: From each of the 92 corns' ears, % of losses was estimated based on an average of damage severity.

Step 2: The loss of each corns' ear was calculated by using a ruler and involved measuring the estimated damage area over the length of corn's ear and (e.g., size of corn ear =25 cm; and with an estimated damage area of 5 cm, Severity is therefore 5 cm/25 cm = 20%).

Farmers' FAW management practices with respect to insecticide usage in Leuk Daek district Kandal province was also recorded.

Efficacy assessment of biopesticides on *Spodoptera frugiperda*

Biological control agents such as Neem oil and the entomopathogenic fungus *Beauveria bassiana*, and *Bacillus thuringiensis* (Bt) toxins are known to be effective at managing the fall armyworm (Josue and Alexi 2022; Apirajkamol et al. 2022; Tay et al. 2022b). Efficacies of *Beauveria bassiana* and Neem oil in managing the FAW were assessed by CARDI twice in August 2021. Solutions of Neem oil and *B. bassiana* were prepared as per manufacturer's instructions (Table 1), followed by dipping the fresh corn leaves into each of these compounds (Figs. 2-4), respectively, before placing single FAW larvae onto these treated leaves. Larvae used were predominantly in the 4th instar larval developmental stage and were collected from Banteay Dek Research Station. For each treatment of Neem Oil or *B. bassiana*, six replications were carried out with FAW larvae exposed to these compounds observed over there consecutive days.

Table 1: Details of manufacturers, stock concentration, and useage instructions for the entomopathogenic fungus *Beauveria bassiana,* and the bioinsecticide Neem Oil. Both the *B. bassiana* and Neem Oil were mad in Indian and supplied by T. Stanes & Company Limited and were purchased on 14/07/2021.

Name	Commercial name	Stock Concentration	Recommended dosage
Beauveria bassiana	BIO POWER	3.5 x 10^8 cfu/g	4Kg/500L H_2O/ha
Neem Oil	NIMBECIDINE	Azadirchtin 0.03%	5mL/L H_2O

Fig. 2: A CARDI researcher performing experiments involving dipping fresh maize leaves into Neem Oil prior to placing single FAW larvae onto these treated leaves (Photo: Sathya K, 27/07/2021 CARDI).

Fig. 3: Neem Oil-treated maize leave with single S. frugiperda larva in each container being monitored for mortality/survival at CARDI (Photo credit: Sathya K, 27/07/2021 CARDI)

Fig. 4: caption please, include also photo credit (Sathya K, 05/08/2021 CARDI)

11.2.3 Results

Severity and Incidence of FAW damage

Based on 100 plants/field with three replications in each district, results on damage incidence and severity showed that among the three provinces, only one province in the Pouk district did not register crop damage by FAW, while maize crop from two provinces of Kampong Cham, and especially in Tbong Khmum, were heavily impacted by the insect (Table 2, Figs. 5 and 6).

Table 2: Severity and incident of FAW on farmers' corn fields in three provinces during the first week of August, 2019.

Province	District	Incidence (%)	Severity (%)
Kampong Cham	Chamka Leu	92	22
Tbong Khmum	Tbong Khmum	25	85
Siem Reap	Pouk	0	0

Fig. 5: Damaged corn from Tbong Khmum district, Tbong Khmum province **(Photo credit:** Sathya Khay, CARDI, 30 July 2019)

Fig. 6: Damaged corn from Chamkar Leu district, Kampong Cham Province. (**Photo credit:** Sathya Khay, CARDI, 31 July 2019).

Two additional evaluations were carried out in June and in November, 2020, in the farmers' corn fields in five districts of the Battambang province, one district in Kampong Cham province (Fig. 7), and two districts in Tbong Khmum province. 100 plants per field with three replications were selected for the assessment in each district. The evaluation was conducted on the third week of June 2020 for Battambang province, the final week of November 2020 for Kampong Cham and Tbong Khmum provinces when the maize plants were about one month old (8 leaves). The results from the assessment indicated that the maize plant and leaf damage incident in the eight districts (three provinces) ranged from 6 to 68% respectively (Table 3).

Fig. 7: Collaborative inspection from CARDI and YAAS of maize crop damage caused by *Spodoptera frugiperda* in Chamkar Leu (Photo by: Kong Sokvisal; Plant Protection Division of CARDI, 31 July 2021).

Table 3: The incidence of corn leaves and plants damage cause by the *Spodoptera frugiperda* in eight districts from Battam Bang, Kampong Cham, and Tbong Khmum provinces in June and in November, 2020.

Province	District	Leaf Incidence (%)	Plant Incidence (%)	Assessment Date
Battam Bang	Komrieng	33	50	15th - 21st June, 2020
	Phnom Proek	41	68	15th - 21st June, 2020
	Borvel	6	20	15th - 21st June, 2020
	Banon	13	36	15th - 21st June, 2020
	Ratanak Mondul	16	46	15th - 21st June, 2020
Kampong Cham	Kampong Siem	8	21	24th - 30th November, 2020
Tbong Khmum	Tbong Khmum	10	23	24th - 30th November, 2020
	Krouch Chmar	27	37	24th - 30th November, 2020

FAW management practices by Cambodian farmers

Farmers at Leuk Daek district Kandal province (Figs. 8-11) informed CARDI that they used insecticides to manage the FAW, with most of them being introduced to pesticides such as Cypermethrin, Abamectin, Emamectin benzoate, and Imidacloprid, by pesticide shop retailers near their village. The farmers were however unclear with respect to the dosage to use, frequencies and best time of application, as well as calibration of application. Importantly, no safety precautions (e.g., protective clothing, eye protection, face masks) were used when applying the insecticides on the maize crops.

Fig. 8: Field survey for FAW and maize damage at the Banteay Dek Agriculture Research Station in Leuk Daek district Kandal province (Photo by PPO/CARDI; 16/07/2021)

Fig. 9: FAW damaged maize crop at Banteay Dek Agriculture Research Station in Leuk Daek district Kandal province (Photo by PPO/CARDI, 16/07/2021).

Fig. 10: A FAW larva feeding on maize crop at Banteay Dek Agriculture Research Station in Leuk Daek district Kandal province (Photo by PPO/CARDI, 16/07/2021).

Fig. 11: Sample collection and assessment of FAW damage on maize in **Banteay Dek Agriculture Research Station,** Leuk Daek district Kandal province **(**Photo by PPO/CARDI, **16 July 2021**).

Assessment of Neem Oil and *Beauveria bassiana* entomopathogenic fungus on FAW

For each treatment of Neem Oil or *B. bassiana* that involved six replications each, no larval mortality was recorded for each treatment for the biopesticide Neem Oil over the three experimental days. For treatments using *B. bassiana*, no sign of fungal growth was observed and larvae did not appeared to have been affected either. CARDI researchers planned to repeat the *B. bassiana* exposure experiment either in glass houses or under field conditions, and will involve collecting FAW larvae and placing them in corn ears (if these corn were not naturally infested), follow by spraying of the fungal biocontrol agent and monitor for fungal infection on FAW. The planned experiments will also include both control and treatment trials, and will involve longer observational period (i.e., from 3 days to 7 days).

11.2.4 Discussion

This report provided initial field survey results from four provinces of Cambodia relating to the extent of maize crop damage caused by the new exotic lepidopteran pest *Spodoptera frugiperda*, as well as infestation levels of this pest between August 2019 and July 2020. The report also detailed farmers' FAW management options, and included some initial findings of the efficacies from biopesticide and biocontrol trials carried out by CARDI. FAW infestation incidence in Chamka Leu District of Kampong Cham Province in 2019 was high at 92% although the damage (i.e., severity) level was only at 22% (Table 2). In the Kampon Siem District of Kampong Cham Province in November 2020 however, the pest infestation incidence was found to be lower with 8% and 21% recorded for maize leaf and plant, respectively (Table 3). In Tbong Khmum Districrt, while only 25% incidence was recorded in August 2019, maize damage was observed at a staggering 85%, suggesting that almost all maize was destroyed of the 25% incidence (Table 3). In June 2020, pest infestation levels in five district of Battam Bang Province ranged from 20% to 68%.

Variability to infestation rates across the landscape in Cambodia is also evident, with Pouk district of the Siem Reap Province recorded no infestation during the August 2019 field survey, and 25% infestation in Tbong Khmum Province that borders Vietnam. However, in Chamka Leu district of Kampong Cham Province which is towards the centre of Cambodia (Fig. 1), it recorded high infestation rate at 92% (Table 1). Population dynamics of the FAW in Cambodia therefore appeared highly variable between 2019 and 2020. The role of insecticide usage between districts and how this could affect pest prevalence is not known. Furthermore, whether these populations also shared the same genetic background of if they each have unique genetic composition as reported between closely located populations in China (e.g., see Jiang et al. 2022) or in Malaysia (e.g., see Rane et al. 2022a) is also at present unknown.

Farmers appeared to be managing this new invasive pest with chemical insecticides such as Cypermathrin, Abamectin, Emamectin benzoate, and imidaclopride. While Bt was available pre-2020, it is currently not available because few farmers were willing to use it, as Bt products were perceived and/or believed to have lower efficacies against lepidopteran pests. Factors such as the introduction of other insecticides elsewhere across the country at the same time, and the ease to purchase these chemical products, have also contributed to the limited up-take of Bt products as alternative control options against the FAW in Cambodia (i.e., no farmers interviewed reported to have used these compounds). Despite the widespread usage of chemical insecticides, there is an urgent need to educate farmers on responsible usage of these chemicals to manage the fall armyworm, since farmers expressed a lack of knowledge on how much of each insecticide to use and how often to apply the chemicals to achieve effective management of *S. frugiperda*. Issues with equipment and dosage calibration, as well as lack of precaution for personal safety with respect to exposure to the chemicals are also main concerns.

Development of resistance to insecticides in the fall armyworm is a valid concern given that native populations of FAW in the Americas are known to be resistant to diverse insecticidal compounds (Carvalho et al. 2013, Fatoretto et al. 2017, Banerjee et al. 2017, Flagel et al. 2018), while resistance alleles such as to pyrethroids (Zhang et al. 2020), to organophosphate (e.g., Guan et al. 2021; Bauventura et al. 2020a; Tay et al. 2022b), and to diamide (Lv et al 2021) have been detected in various invasive populations. While in Pouk District (Siem Reap Province; Table 1) no maize crop damage was reported and was suggested to be likely due to heavy usage of the Cypermethrin insecticide, prolong and widespread usage of this insecticide could lead to development of resistance, especially if population migration is occurring and involving mixing of populations with pyrethroid resistance alleles from neighbouring countries. There is a need to better understand the insecticide profiles of FAW in Cambodia using bioassay protocols such as the ones developed by the GRDC/ACIAR funded SEA FAW biosecurity preparedness project, as well as genomic characterisation of resistance genes via whole genome sequencing methods as demonstrated by Guan et al. (2020) and Tay et al. (2022b).

In Cambodia, FAW control management practices appeared to differ between farmers, highlighting the need for a national coordinated approach especially for chemical insecticides that needs to include educating both farmers and extension officers. Exploring IPM strategies that involves the use of entomopathogenic fungi (e.g., *B. bassiana*), biopesticides (e.g., Neem Oil), understanding the diversity of beneficial insects especially through molecular diagnostics approach (e.g., Otim et al. 2021), and Bt products (Tay et al. 2022b) will require appropriate training of local researchers with international support, including development of standardised biocontrol bioassay protocols (e.g., Tay et al. 2022b; Apirajkamol et al. 2022). While initial population genomic analyses of FAW populations across SEA have provided valuable insights to understanding biosecurity vulnerability at regional scale (Rane et al. 2022a; Tay et al. 2023), this is however not the case for various SEA countries such as for Indonesia and for Cambodia, despite CARDI's effort to share *S. frugiperda* material with international collaborators. This further highlights the challenge (e.g., legislations preventing easy sharing of biological material) faced by Cambodian scientists to obtain relevant population-specific genetic and biological knowledge that is urgently needed to assist local farmers in managing this pest and to improve their livelihood.

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11.3 Appendix 3: *Spodoptera frugiperda* VGSC & ACE-1 Resistance Allele Frequencies (from Tay et al. 2021a)

		VGSC [L1014F]				VGSC [L932F]			VGSC [T929I]				AChE-1 [A201S]				AChE-1 [G227A]				AChE-1 [F290V]					
Bargraph ID	Country (State/Province)	N	S (%)	R (%)		N	S (%)	R (%)	N	S (%)	R (%)		N	S (%)		R (%)	N	S (%)	R (%)		N	S (%)		R (%)	References	Comments
	Brazil	140	1	0		16.7	1	0	143	1	0		147	0.94		0.06	161	0.71	0.29		127	0.78		0.22	Bauventura et al. (2020) Insects 11(8), 10.3390/insects11080545	
	Brazil (Goias)	14	1	0		14	1	0	14	1	0		14	0.86		0.14	14	0.64	0.36		14	0.79		0.21	Guan et al. (2021) Insect Science 28(3), 627-638	
	French Guiana	3	1	0		3	1	0	3	1	0		3	1		0	3	1	0		3	0.83		0.17	Yainna et al. (2021) Insects 12, 468	
	Peru	16	1	0	0	16	1	0	16	1	0		16	0.62		0.38	16	0.81	0.19		16	0.44		0.56	This study	
	Mexico	26	1	0		26	1	0	26	1	0		26	1		0	26	1	0		26	0.465		0.535	Yainna et al. (2021) Insects 12, 468	
	Guadeloupe	4	1	0		4	1	0	4	1	0		4	1		0	4	1	0		4	1		0	Yainna et al. (2021) Insects 12, 468	
	Puerto Rico	70	1	0		70	1	0	70	1	0		70	1		0	70	1	0		29	0.09		0.91	Bauventura et al. (2020) Insects 11(8), 10.3390/insects11080545	
	Puerto Rico	15	1	0		15	1	0	15	1	0		15	1		0	15	0.97	0.03		15	0.37		0.63	This study	
	USA (Mississippi)	18	1	0		18	1	0	18	1	0		18	0.94		0.06	18	0.94	0.06		18	0.67		0.33	This study	
	USA (Florida)	20	1	0		20	1	0	20	1	0		20	0.97		0.03	20	0.92	0.08		24	0.71		0.29	This study	
	Africa																									
1	Benin	39	1	0		39	1	0	39	1	0		39	0.79		0.21	39	1	0		39	0.2		0.8	Yainna et al. (2021) Insects 12, 468	
2	Uganda	15	1	0		15	1	0	15	1	0		15	0.97		0.03	15	1	0		15	0.43		0.57	Rane et al. (2022)	
3	Kenya	76	1	0		75	1	0	75	1	0		76	0.94		0.06	76	1	0		76	0.5		0.5	Bauventura et al. (2020) Insects 11(8), 10.3390/insects11080545	
4	Tanzania	1	1	0		1	1	0	1	1	0		1	1		0	1	1	0		1	0.5		0.5		
5	Zambia (Lasuka) reseq	2	1	0		2	1	0	2	1	0	†	2	0.91		0.09	2	1	0	†	2	0.41		0.59	Zhang et al. (2020) Mol Ecol Res 20(6), 1682-1696	† Values as reported that combined Zambia (n=2), Malawi (n=2), and China cities (n= 103)
	Malawi	16	1	0		16	1	0	16	1	0		16	0.91		0.09	16	1	0		16	0.56		0.44	Guan et al. (2021) Insect Science 28(3), 627-638	
	Malawi (Bvumbwe) reseq	2	1	0		2	1	0	2	1	0	†	2	0.91		0.09	2	1	0	†	2	0.41		0.59	Zhang et al. (2020) Mol Ecol Res 20(6), 1682-1696	† Values as reported that combined Zambia (n=2), Malawi (n=2), and China cities (n= 103)
6	Malawi AVERAGE													0.91		0.09		1	0			0.54		0.46		Average values used
	Asia																									
1	India	12	1	0		12	1	0	12	1	0		12	0.92		0.08	12	1	0		12	0.38		0.62	Rane et al. (2022)	
	Myanmar (Aungban)	3	1	0		3	1	0	3	1	0		3	1		0	3	1	0		3	0.5	1.5	0.5	Rane et al. (2022)	
	Myanmar (Kimpoungtaung)	3	1	0		3	1	0	3	1	0		3	0.67		0.33	3	1	0		3	0.5	1.5	0.5	Rane et al. (2022)	
	Myanmar (Kengtung)	2	1	0		2	1	0	2	1	0		2	0.5		0.5	2	1	0		2	0.75	0.5	0.25	Rane et al. (2022)	
	Myanmar (Tatkone)	3	1	0		3	1	0	3	1	0		3	0.67		0.33	3	1	0		3	0.67	0.99	0.33	Rane et al. (2022)	
	Myanmar (Wax)	3	1	0		3	1	0	3	1	0		3	0.67		0.33	3	1	0		3	0.67	0.99	0.33	Rane et al. (2022)	
	Myanmar (YZ)	3	1	0		3	1	0	3	1	0		3	0.67		0.33	3	1	0		3	0.33	2.01	0.67	Rane et al. (2022)	
2	Myanmar AVERAGE													0.71		0.29		1	0			0.56		0.44		Average values used
	Laos (Vientiane)	5	1	0		5	1	0	5	1	0		5	0.9	4.5	0.1	5	1	0		5	0.6	3	0.4	Rane et al. (2022)	
	Laos (Champasak)	5	1	0		5	1	0	5	1	0		5	0.8	4	0.2	5	1	0		5	0.5	2.5	0.5	Rane et al. (2022)	
	Lao (Xiengkhouang)	10	1	0		10	1	0	10	1	0		10	0.75	7.5	0.25	10	1	0		10	0.5	5	0.5	Rane et al. (2022)	
3	Laos AVERAGE													0.8		0.2		1	0			0.53		0.47		Average values used
	Vietnam	11	1			11	1	0	11	1	0		11	0.91		0.09	11	1	0		11	0.55		0.45	Tay et al. GRDC Report (2021)	Not used; data based on Whole genome sequencing
4	Vietnam (01-Kim Bol)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.96		0.04	15	1	0		15	0.64		0.36	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
5	Vietnam (02-Nam Dan)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.83		0.17	15	1	0		15	0.41		0.59	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
6	Vietnam (03-Tho Xuan)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.89		0.11	15	1	0		15	0.42		0.58	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
7	Vietnam (04-Phu Tho)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.96		0.04	15	1	0		15	0.46		0.54	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
8	Vietnam (05-Bao Lam)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.89		0.11	15	1	0		15	0.36		0.64	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
9	Vietnam (06-Bao Loc)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.77		0.23	15	1	0		15	0.52		0.48	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
10	Vietnam (07-Buon Don, Tan Hoa)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.92		0.08	15	1	0		15	0.26		0.74	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
11	Vietnam (08-Eakar)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.9		0.1	15	1	0		15	0.46		0.54	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
12	Vietnam (09-Buon Don, EaNoul)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.87		0.13	15	1	0		15	0.3		0.7	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
13	Vietna (10-Cao Lanh)		n/a	n/a			n/a	n/a		n/a	n/a		15	0.85		0.15	15	1	0		15	0.4		0.6	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
14	Malaysia (Penang)	9	1	0		9	1	0	9	1	0		9	0.83		0.17	9	1	0		9	0.67		0.33	Rane et al. (2021)	
15	Malaysia (Johore)	10	1	0		10	1	0	10	1	0		10	0.8		0.2	10	1	0		10	0.55		0.45	Rane et al. (2021)	
16	Malaysia (Kedah)	10	1	0		10	1	0	10	1	0		10	1		0	10	1	0		10	0		1	Rane et al. (2021)	
17	Indonesia	110	0.99	0.01		88	1	0	88	1	0		85	0.89		0.11	86	0.92	0.08		86	0.48		0.52	Bauventura et al. (2020) Insects 11(8), 10.3390/insects11080545	
	Papua New Guinea (Medang)	16	1	0		16	1	0	16	1	0		16	0.94		0.06	16	1	0		16	0.56	8.96	0.44	This study	
	Papua New Guinea (Yule Is. Junction)	1	1	0		1	1	0	1	1	0		1	1		0	1	1	0		1	0		1	This study	
18	Papua New Guinea AVERAGE													0.94		0.06		1	0			0.53		0.47		Average values used
19	Philippines (Lipa)	20	1	0		20	1	0	20	1	0		19	0.98		0.02	20	1	0		19	0.58		0.42	Rane et al. (2021)	
	South Korea (MF)	7	1	0		7	1	0	7	1	0		7	1		0	7	1	0		7	1		0	Rane et al. (2021)	

	South Korea (MY)	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	Rane et al. (2021)	
	South Korea (Gr, Mn, MA)	3	1	0	3	1	0	3	1	0	3	0.83	0.17	3	1	0	3	1	0	Rane et al. (2021)	
20	Korean AVERAGE											0.96	0.04		1	0		1	0		Average values used
	China																				
	China (HIA006)										1	1	0	1	1	0	1	0	1	Tay et al. GRDC Report (2021)	Not used
	China (21 provinces) *PCR@F290V	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a	173	0.5	0.5	Zhang et al. (2020) Mol Ecol Res 20(6), 1682-1696	Not used
	China (50 cities, 16 provinces) reseq	103	1	0	103	1	0	103	1	0	† 103	0.91	0.09	103	1	0	† 103	0.41	0.59	Zhang et al. (2020) Mol Ecol Res 20(6), 1682-1696	Not used; † Values as reported that combined Zambia (n=2), Malawi (n=2), and China cities (n=103)
	China	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	0	1	Yainna et al. (2021) Insects 12, 468	Not used
1	China (Cangyuan CY, Yunnan)	20	1	0	20	1	0	20	1	0	20	1	0	20	1	0	20	0.33	0.67	Guan et al. (2021) Insect Science 28(3), 627-638	
2	China (Ximeng XP, Yunnan)	15	1	0	15	1	0	15	1	0	15	0.77	0.23	15	1	0	15	0.57	0.43	Guan et al. (2021) Insect Science 28(3), 627-638	
3	China (Yuanjiang YJ, Yunnan)	14	1	0	14	1	0	14	1	0	14	0.93	0.07	14	1	0	14	0.5	0.5	Guan et al. (2021) Insect Science 28(3), 627-638	
4	China (Dehong, Yunnan)	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a	12	0.67	0.33	12	1	0	14	0.68	0.32	Zhao et al. (2020) Pesticide Biochem Phsiol 168, 104623	
5	China (Anlong AL, Guizhou)	6	1	0	6	1	0	6	1	0	6	0.9	0.08	6	1	0	6	0.5	0.5	Guan et al. (2021) Insect Science 28(3), 627-638	
6	China (Jiangmen JM, Guangdong)	10	1	0	10	1	0	10	1	0	10	0.9	0.1	10	1	0	10	0.4	0.6	Guan et al. (2021) Insect Science 28(3), 627-638	
7	China (Zengcheng ZC, Guangdong)	10	1	0	10	1	0	10	1	0	10	1	0	10	1	0	10	0.35	0.65	Guan et al. (2021) Insect Science 28(3), 627-638	
8	China (Dean DA, Jiangxi)	9	1	0	9	1	0	9	1	0	9	1	0	9	1	0	9	0.33	0.67	Guan et al. (2021) Insect Science 28(3), 627-638	
9	China (Jingzhou JZ, Hubei)	12	1	0	12	1	0	12	1	0	12	0.96	0.04	12	1	0	12	0.54	0.46	Guan et al. (2021) Insect Science 28(3), 627-638	
10	China (Jingzhou, Hubei)	20	1	0	20	1	0	20	1	0	20	1	0	20	0.97	0.03	20	0.39	0.61	Guo et al. 2020 ACTA Entomol Sinica 63(5), 582-589	
11	China (Wuxue, Hubei)	20	1	0	20	1	0	20	1	0	20	0.97	0.03	20	0.82	0.18	20	0.72	0.28	Guo et al. 2020 ACTA Entomol Sinica 63(5), 582-589	
12	China (Xianning, Hubei)	20	1	0	20	1	0	20	1	0	20	1	0	20	0.9	0.1	20	0.3	0.7	Guo et al. 2020 ACTA Entomol Sinica 63(5), 582-589	
13	China (Huanggang, Hubei)	20	1	0	20	1	0	20	1	0	20	0.95	0.05	20	0.9	0.1	20	0.58	0.42	Guo et al. 2020 ACTA Entomol Sinica 63(5), 582-589	
14	China (Anqing AQ, Anhui)	12	1	0	12	1	0	12	1	0	12	1.0	0.04	12	1	0	12	0.4	0.6	Guan et al. (2021) Insect Science 28(3), 627-638	
15	China (Jingxian, Anhui)	17	1	0	17	1	0	17	1	0	15	1	0	15	1	0	15	0.33	0.67	Zhao et al. (2020) Pesticide Biochem Phsiol 168, 104623	
16	China (Dongtai, Jiangsu)	19	1	0	19	1	0	19	1	0	25	0.78	0.22	25	1	0	25	0.26	0.74	Zhao et al. (2020) Pesticide Biochem Phsiol 168, 104623	
	Australia																				
1	Australia (Broome, WA)	10	1	0	10	1	0	10	1	0	11	0.95	0.05	11	1	0	11	0.18	0.82	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
2	Australia (Kununurra, WA)	19	1	0	19	1	0	19	1	0	19	1	0	19	1	0	19	0.45	0.55	This study + Rane et al. (2022)	
3	Australia (Kununurra KB1, WA)	8	1	0	8	1	0	8	1	0	8	0.94	0.06	8	1	0	8	0.44	0.56	Rane et al. (2022)	
4	Australia (Kununurra KB2, WA)	7	1	0	7	1	0	7	1	0	7	0.93	0.07	7	1	0	7	0.71	0.29	Rane et al. (2022)	
5	Australia (WA108)		n/a	n/a		n/a	n/a		n/a	n/a	15	1	0	15	1	0	15	0.30	0.70	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
6	Australia (WA203)		n/a	n/a		n/a	n/a		n/a	n/a	15	1	0	15	1	0	15	0.50	0.50	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
7	Australia (WA207)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.91	0.09	15	1	0	15	0.45	0.55	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
8	Australia (WA210)		n/a	n/a		n/a	n/a		n/a	n/a	15	1	0	15	1	0	15	0.2	0.8	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
9	Australia (WA301)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.95	0.05	15	1	0	15	0.33	0.67	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
10	Australia (WA302a)		n/a	n/a		n/a	n/a		n/a	n/a	15	1	0	15	1	0	15	0.6	0.4	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
11	Australia (WA302)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.89	0.01	15	1	0	15	0.26	0.74	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
12	Australia (WA303)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.98	0.02	15	1	0	15	0.13	0.87	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
13	Australia (WA307)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.75	0.25	15	1	0	15	0.45	0.55	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
14	Australia (WA309)		n/a	n/a		n/a	n/a		n/a	n/a	15	1	0	15	1	0	15	0.28	0.72	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
15	Australia (WA402)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.97	0.03	15	1	0	15	0.35	0.65	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
16	Australia (WA403)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.83	0.17	15	1	0	15	0.55	0.45	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
17	Australia (WA406)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.9	0.1	15	1	0	15	0.25	0.75	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
18	Australia (WA408)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.76	0.24	15	1	0	15	0.68	0.32	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
19	Australia (WA410)		n/a	n/a		n/a	n/a		n/a	n/a	15	0.96	0.04	15	1	0	15	0.4	0.6	Tay et al. GRDC Report (2021)	Data from amplicon metabarcoding
20	Australia (Ord Valley, WA)	14	1	0	14	1	0	14	1	0	14	0.96	0.04	14	1	0	14	0.25	0.75	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
21	Australia (Bluey's Farm, NT)	6	1	0	6	1	0	6	1	0	6	1	0	6	1	0	6	0.67	0.33	Rane et al. (2022)	
22	Australia (Katherine, Tipperary, Douglas Daly, NT)	3	1	0	3	1	0	3	1	0	18	0.92	0.08	18	1	0	18	0.45	0.55	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
23	Erub Is. Australia	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	0.5	0.5	Rane et al. (2022)	
24	Australia (Lakeland, Qld)	11	1	0	11	1	0	11	1	0	10	0.9	0.1	10	1	0	10	0.55	0.45	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
25	Australia (Walkamin, Qld)	19	1	0	19	1	0	19	1	0	20	0.82	0.18	20	1	0	20	0.53	0.47	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
26	Australia (Walkamin 'unsprayed', Qld)	19	1	0	19	1	0	19	1	0	19	0.87	0.13	19	1	0	20	0.95	0.05	Rane et al. (2022)	
27	Australia (Walkamin 'early sprayed', Qld)	9	1	0	9	1	0	9	1	0	9	0.89	0.11	9	1	0	9	1	0	Rane et al. (2022)	
28	Australia (Walkamin 'mango', Qld)	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	Rane et al. (2022)	
29	Australia (Strathmore, Qld)	30	1	0	30	1	0	30	1	0	30	0.93	0.07	30	1	0	30	1	0	This study	
30	Australia (Croydon, Qld)	2	1	0	2	1	0	2	1	0	2	0.75	0.25	2	1	0	2	0.75	0.25	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
31	Australia (Burdekin, Qld)	5	1	0	5	1	0	5	1	0	5	0.7	0.3	5	1	0	5	0.6	0.4	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
32	Australia (Burdekin, Qld)	30	1	0	30	1	0	30	1	0	30	0.9	0.1	30	1	0	30	1	0	Rane et al. (2022)	
33	Australia (Bowen, Qld)	10	1	0	10	1	0	10	1	0	10	0.85	0.15	10	1	0	10	0.8	0.2	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
34	Australia (Mackay, Qld)	7	1	0	7	1	0	7	1	0	7	0.71	0.29	7	1	0	7	0.71	0.29	Rane et al. (2022)	
35	Australia (Wee Waa, NSW)	8	1	0	8	1	0	8	1	0	8	1	0	8	1	0	8	0.63	0.37	Rane et al. (2022)	
36	Australia (North West, NSW)		n/a	n/a		n/a	n/a		n/a	n/a	9	0.94	0.06	9	1	0	9	0.33	0.67	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
37	Australia (North Coast, NSW)		n/a	n/a		n/a	n/a		n/a	n/a	14	0.86	0.14	14	1	0	14	0.54	0.46	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
38	Australia (Central West, NSW)		n/a	n/a		n/a	n/a		n/a	n/a	7	0.86	0.14	7	1	0	7	0.57	0.43	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	
39	Australia (Riverina, NSW)		n/a	n/a		n/a	n/a		n/a	n/a	3	1	0	3	1	0	3	0.83	0.17	Nguyen et al. (2021) Austral Entomol, https://doi.org/10.1111/aen.12570	

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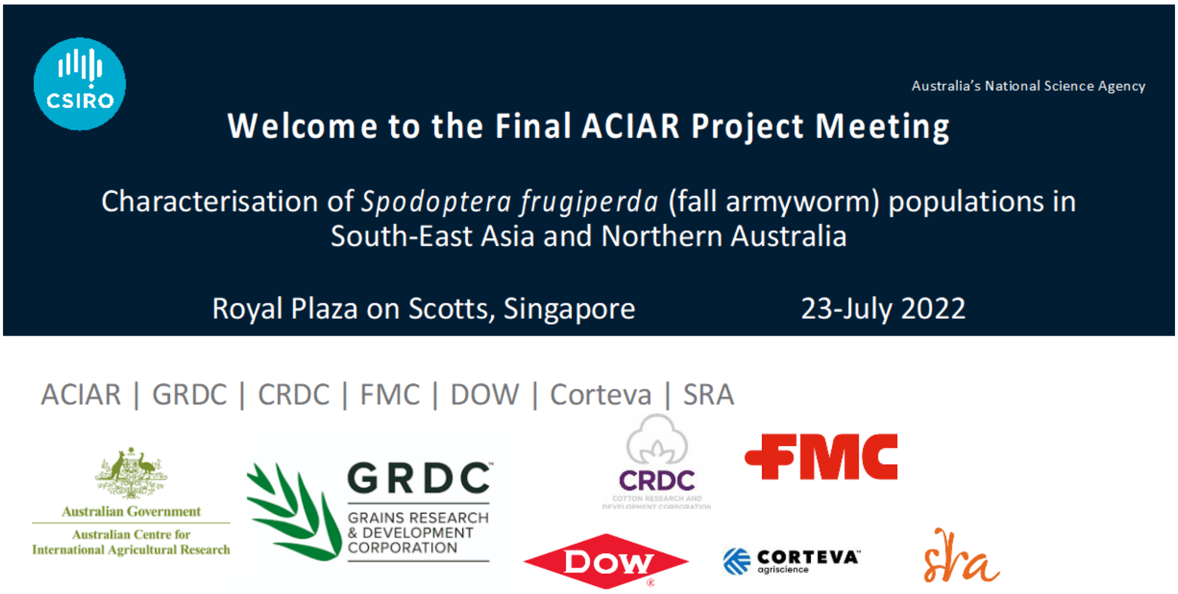
11.4 Appendix 4: Comparisons of *Spodoptera frugiperda* (FAW) Management Practices between Uganda & Southeast Asia Nations

FAW control methods	Categories	Uganda (A Kalyebi)	References	Uganda (NARO)	References	Malaysia	References	Myanmar	References	Philippines	References	Cambodia	References	Vietnam	References	Laos	References	Indonesia	References
intercropping	Cultural	yes, mono- vs. poly-cultures	Andrew Kalyebi (AK) pers comm.	Maize intercropped with food legumes, soybean, goundut	Hailu et al. 2018, Agronomy Jounal 110(6), 2513-2522			No	CABI SEA							Yes, maize with cucurbits, ground beans, pumpkins, cassava	PPC KC		
crop rotation	Cultural	yes, including soybeans, goundnuts, potatoes, cassava, sorghum	AK			Yes, information on crop rotation and its associated benefits prepared and shared	Mazidah Binti Mat (MBM), Malaysia Agicultural Research and Development Insitute (MARDI)	rice-Veg-maize	CABI SEA							Yes, rice	PPC KC		
Crop diversification	Cultural									Yes, prevention/avoidance	Divina Amalin, De La Salle University (DLSU)								
Synchronous planting	Cultural					Yes, information on simultaneous planting and its rationale and associated benefits prepared and shared	MARDI			Yes, prevention/avoidance	DLSU								
field sanitation	Cultural	Yes, weeding, land preparation, refuse clearing	AK	yes, weed manipulation	NARO MHO					Yes, prevention/avoidance	DLSU								
Soil penetration	Cultural	yes, via regular weeding	AK					deep (>20cm)	CABI SEA					Yes, target pupae	Nguyen Van Liem, Plant Protection Research Institute (PPRI)				
GM maize/resistant varieties/tolerant variety	cultural			Yes, trails in progress	NARO MHO									yes, as long term solutions	PPRI	Yes, information on resistance variety presented	PPC KC		
information technologies, remote sensing	technical			Yes, mobile phones	NARO MHO									solutions, drones deployment	PPRI				
flooding treatment	Cultural													yes, flooding at pupal stage					
Applying alternative substances	Cultural	yes, animal/human urine, plant extracts (Aleo vera, tobacco chili pepper, Lantana camara, Neem in combination with ash	AK	yes, various substances including pepper, ash, paraffin	NARO MHO														
Early planting	Cultural			yes	NARO MHO														
Organic fertiliser	Cultural	yes, cow manure as fertiliser	AK																

FAW control methods	Categories	Uganda (A Kalyebi)	References	Uganda (NARO)	References	Malaysia	References	Myanmar	References	Philippines	References	Cambodia	References	Vietnam	References	Laos	References	Indonesia	References
Trap crops/Trap plants	IPM/Cultural			Yes	NARO MHO					Yes, early detection	DLSU								
field inspection/couting/survey	IPM/Cultural	yes, validate farmers responses	AK			Yes,farmer's field inspections	MARDI, Departmetn of Agriculture (DoA)			Yes, early detection/surveillance	DLSU			yes, field monitoring; early warning	PPRI				
Use pest attractants	IPM/Cultural			Yes, lures, pheromone traps	NARO MHO	yes, pheromone (Fero Ttrap) and UV light traps to monitor adult FAW populations in sweet corn fields	MARDI, DoA			Yes, early detection	DLSU			Yes, pheromones, 'sweet & sour bait traps	PPRI	Yes, use of pheromone traps, molasses	PPC KC		
'food spray'	IPM/cultural													yes, rice flour, yeast,	PPRI				
Grow hedges and flowers	IPM			Yes, as 'push-pull'	NARO MHO			No	CABI SEA										
Plant quarantine regulation	IPM									Yes, prevention/avoidance	DLSU								
Identification of hotspots	IPM									Yes, surveillance	DLSU								
Parasitoids/biological control	IPM	Not actively practiced by farmers	AK	Yes, molecular characterisation; other documentation	NARO MHO; Otim et al. 2019	Yes, monitored in trail plots	MARDI, MDM			yes, detected	CABI SEA, DLSU	yes, trialed (*T. remus*)	K. Sathya CARDI	Yes, parasitoids, earwigs, spiders, ladybird, NPV, EPF, nematodes	PPRI	Yes, farmers tried using stink bugs	PPC KC	yes, *Telenomus*, *Trichogramma*	Universitas Gadjah Mada (UGM), Wahyuningsih et al. 2022 JPTI
Biopesticides	IPM					yes, Fawligen SfMNPV trails conducted	CABI SEA; Faheem Muhammad (FM)			yes, BT, B. subtilis B. mayloliquefaciens	DLSU	yes, trialed (see report)	K. Sathya CARDI	Yes, Bt, Neem	PPRI			Yes, Bt	UGM
Awareness campaign	IPM			Yes, National taskforce formed, management material produced and distributed, local language material distributed, radio talk shows	Naro MHO	Yes, field visits to farmers' farms attacked by FAW; Sharing knowledge and expertise with farmers on FAW management, guides and information leaflets provided	MARDI, DoA			Yes, prevention/avoidance	DLSU			Yes, workshop, guides, training	PPRI	Yes, ToT provided by FAO, local governemnt and grower association	PPC KC		
Pest advisory	IPM	Yes, including advice on insecticide applications	AK	Yes, National FAW control strategy and acrtion plan	Naro MHO	yes, on insecticide usage at different DAP in maize crops	MARDI			Yes, prevention/avoidance	DLSU								
Push-Pull technology	IPM	Yes	AK	yes	NARO MHO	Yes, information on push-pull technique prepared and shared	DoA												
physical	Cultural/ mechenical	yes, manual destruction of egg masses	AK	Yes, manual egg mass destruction	NARO MHO	Yes, pictorial guide provided on how to conduct field scouting activities	DoA			Yes, suppression	DLSU			yes, manual egg mass destruction	PPRI				
Pesticides	chemical	yes, but with varying degrees of successes/failures	AK	yes, identify and recommend insecticides for FAW control	NARO MHO	Yes, IPM included a component of insecticide rotation between Bt, Emamectin benzoate and chlorantraniliprole to target specific plant growth stages	MARDI			Yes, suppression	DLSU			yes, seed treatment, insecticide spray	PPRI	Yes, on large and small-scale farms	PPC KC		

11.5 Appendix 5: Final Project Partners Meeting: Characterisation of *Spodoptera frugiperda* (Fall armyworm) populations in South-East Asia and Northern Australia

The final partner meeting was organised by CSIRO and held at the Royal Plaza on Scotts Hotel in Singapore on 23rd July 2022, from 8:50am to 5:30pm, and involved representatives from the GRDC, ACIAR, CSIRO Business Development and Global, ASEAN FAW Action Plan, and project partners from Philippines, Vietnam, Laos PDR, Cambodia, Malaysia, Indonesia, Uganda, and CSIRO.



ACIAR (co-funded with GRDC) - Final Project Partners Meeting: Characterisation of *Spodoptera frugiperda* (fall armyworm) populations in South-East Asia and Northern Australia

23rd July 2022. Royal Plaza on Scotts, 25 Scotts Road Singapore 228220.

Pre-meeting dinner: Straitskitchen. Lobby Level, Grand Hyatt Singapore.

Dress code: Smart Casual.

Date | Time: 22-July, 2022 | 6:30 pm

Meeting Program for 23rd July 2022

		Speaker	session chair	Title / Comment
8:50 - 8:55	CSIRO Welcome	Wee Tek Tay	Andrew Kalyebi	
8:55- 9:00	ACIAR Welcom	Eric Huttner		
9:00- 9:10	GRDC	Callum Fletcher		
9:10 - 9:30	Malaysia	Sathis Sri Tanarajoo		Highlights of FAW Bioassays, Management and Awareness in South East Asia
9:30 - 9:50	Cambodia	Khay Sathya		Overview and Challenges of FAW studies in Cambodia
9:50 - 10:10	Philippines	Divina Amalin		Fall Armyworm Invasion and Post-entry Management Efforts in the Philippines
10:10 - 10:50	Morning break			
10:50 - 11:10	Laos	Khonesavanh Chitterath	Siva Annamalai	Toxicity of insecticides on the Fall Armyworm from Laos
11:10 - 11:30	Vietnam	Nguyen Van Leim		Overview of Management and Studying on FAW in Vietnam
11:30 - 11:50	Indonesia	Valentina Aryuwandari		Toxicity of Several Insecticides and Bt Toxins on the Fall Armyworm from Indonesia
11:50 - 12:10	Uganda	Michael Otim		Update on fall armyworm research and management in Ugand
12:10 - 13:40	Lunch			Carousel Restaurant (Royal Plaza on Scotts)
13:40 - 14:00	Uganda	Andrew Kalyebi	Divina Amalin	Farmer perception of Spodoptera frugiperda in Uganda, management practices and potential for transferability
14:00 - 14:20	Australia Bioassays	Wee Tek Tay		Insecticide resistance profiles inferred FAW introduction pathways in Asia-Pacific
14:20 - 14:40	Australia WGS	Rahul Rane		Genetic signatures informed FAW movements in APAC
14:40 - 15:00	ASEAN FAW Action Plan	Alison Watson		
15:00 - 15:45	Afternoon break			
15:45 - 16:00	ASEAN Bioprotection RC	Amelia Fyfield	Alison Watson	
16:00 - 16:30	Future opportunities			breakout in 3 groups in 5/grp
16:30 - 16:40	Group 1 summary			
16:40 - 16:50	Group 2 summary			
16:50 - 17:00	Group 3 summary			
	Closing remarks	Eric / Tek		
18:30	Dinner	Indonesia (Halal meal)		within walking distance (~15 min from the hotel)
		Tambuam Mas		Suggest to meet at hotel foyer at 18:00 (leave at 18:10)
		(Tanglin Shopping Centre #04-10/13)		

CSIRO Australia's National Science Agency 1

Summary of project partners' presentations at the final meeting in Singapore

1. Malaysia: Muhammad Faheem, Sathis Sri Thanarajoo, Sivapragasam Annamalai (CABI Project Team)

Presentation title: Highlights of FAW Bioassays, Management and Awareness in South East Asia

SUMMARY

The CABI team in Malaysia shared the challenges they encountered which included: (i) COVID-19 travel restriction impeded FAW collection from different regions; (ii) encountered FAW rearing difficulties such as issues with FAW diet that required trial and error approaches; (iii) inability to carry out bioassays in a timely manner due to COVID-19 restriction on number of people allowed to be working in close proximity within buildings; (iv) limited knowledge on field populations' insecticide resistance/susceptibility profiles.

CABI-FARM (CABI-IPM plan against FAW for Maize Learning Plot) recommended (i) crop rotation to reduce FAW inoculum, (ii) intercrop maize with compatible and less susceptible crops (e.g., beans, cassava); (iii) plant hedges and flowers along field, and (iv) superficial tillage (< 10cm deep).

- CABI-FARM recommendation is based on current widespread report that the invasive FAW has a feeding preference on maize (e.g., observed in Myanmar), and hedges and flowers are friendly microhabitats that can attract natural enemies of FAW, and tillage to destroy FAW pupae, with shallow tillage to reduce labour, nutrient loss, and to favour soil microfauna.
- CABI is also evaluating bio-based (e.g., bio-pesticides, pheromones) and chemical interventions both in laboratory and in-field settings.

2. Cambodia: Khay Sathya (Plant Protection Division of CARDI)

Presentation Title: Overview and challenges of FAW studies in Cambodia

SUMMARY

COVID-19 pandemic-related challenges impacted the ability to carry out project milestones, including extensive and repeated travel restriction and total lockdown, regular interruptions to electricity supplies, and monsoon and cyclone related floods.

FAW damage level assessed in maize fields from five provinces (Battam Bang; Kampong Cham; Tboung Khmum; Kandal; Siem Reap), with varying levels of pest incidences ranging from 14% - 92%, and damage severity in corn as high as 85% detected in August 2019 from Tbong Khmum Province.

Trialled biopesticides (Neem Oil), entomopathogenic fungi (*Beauveria bassiana*), and beneficial insects (*Telenomus remus*).

- Trials involving Need Oil and *B. bassiana* were unsuccessful (see Appendix 2)
- *Telenomus remus* field trials (100,000 – 150,000/ha released) gave significantly improved plant protection to: (i) leaf damage (32±3% control vs. 19±4% *T. remus*); (ii) corn ear damage (66±3% control vs. 64±2% *T. remus*); (iii) % yield loss (30±4% control vs. 24±5% *T. remus*); (iv) t/ha yield (6.74±1 control vs. 7.44±1 *T. remus*)

3 Philippines: Divina M. Amalin, Judy Ann P. Verbosidad (Biological Control Research Unit, De La Salle University, Manila)

Presentation Title: Fall armyworm invasion and post-entry management efforts in the Philippines

SUMMARY

FAW first reported at Piat, Cagayan, on 20-June, 2019; estimated national damage = 26.61% from 70 provinces (79 total provinces) as at December 2020.

The project encountered challenges including the Taal Volcano eruption and flooding from Typhoon Ulysses, difficulties of establishing laboratory culture due to diet incompatibility that required trial-and-error experimentation to develop most suitable FAW diet

FAW management strategies in the Philippines included implementing pre-emptive measures to prevent spread, including early detection, proactive identification of pest hot-spots through intensified surveillance and monitoring, capacity building of farmers and local technicians to manage early stage of FAW spread, develop contingency measures and actions to contain and manage large scale outbreak, establish support programs (e.g., sustain capacity building, increase awareness in FAW management), engage stakeholders through effective communication using various tools and strategies, and encourage research and development on FAW IPM.

- Insecticide management and susceptibility studies of FAW
- Genetic structure and morphological variation analyses of FAW in the Philippines
- Identification and preliminary evaluation of FAW natural enemies

4. Laos: Ms Khonesavanh Chittarath (Plant Protection Center, DOA, MAFF, Laos)

Presentation Title: Toxicity of insecticides on the fall armyworm from Laos

SUMMARY

Challenges from the Laos project team included lockdown due to the COVID-19 pandemic that complicated sending of samples to Australia for genomic analysis, lack of technical expertise relating to rearing of FAW larvae and laboratory colony maintenance, as well as inexperience with undertaking insecticide bioassay experiments, which is further complicated by language difficulties especially relating to communicating work activities and solving relating technical issues.

Despite these issues samples were successfully sent to CSIRO to contribute to the SEA FAW population genomic study, resistance allele characterisation, as well as successfully completed insecticide bioassays.

5. Vietnam: Nguyen Van Liem, Dao Thi Hang (Plant Protection Research Institute (PPRI); Vietnam Academy of Agricultural Sciences (VAAS), Ministry of Agriculture and Rural Development (MARD)

Presentation Title: Overview of Management and studying on FAW in Vietnam

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT (MARD)
VIETNAM ACADEMY OF AGRICULTURAL SCIENCES (VAAS)
PLANT PROTECTION RESEARCH INSTITUTE (PPRI)

Overview of Management and Studying on FAW in Vietnam





Singapore, 23 July 2022

SUMMARY

Spodoptera frugiperda was recorded for the first time in 2008 on grass fields around Hanoi (Vu 2008; Nguyen and Vu 2009; Pham 2019), with outbreak recorded from early 2019 in northern provinces, followed by central provinces and finally in southern provinces. PPD surveys in 2022 confirmed 58/63 provinces have FAW damages. Across the three years from 2019, 2020, and 2021 however, there has been a decrease in infestation rates, from 7.8% to 2.8% to 1.5%, respectively.

Management solutions undertaken by MARD (consisted of PPD, Provincial ARD Departments, PPRI, VNUA) including workshops, training, leaflets/education material (‘propaganda information’) to guide FAW control to maize growers and technicians/extension officers.

Solutions to manage FAW is divided in ‘present’ and ‘long-term’ solutions.

- Present solutions include: (i) use of resistant varieties (5 GM corn varieties being tested), (ii) seed treatments (Fortenza Duo 480FS (cyantraniliprole+Thiamethoxam) to protect gemination to 3-5 leaf stage), (iii) using sweet and sour bait traps or pheromone traps (as a tool to monitor and control adult FAW), (iv) biological (e.g., *Metarhizium* spp., Bt, NPV, entomopathogenic nematodes, earwigs, spiders, ladybird beetles, *Telenomus*, *Trichogramma*) and manual methods (e.g., flooding targeting pupae; removing egg masses; soil tillage), and (v) chemical methods (e.g., monitor larval density, feeding damage signs, and maize growth stage to determine action threshold for pesticide applications using Bt, Spinetoram, Indoxacarb, Lufenuron, Emamectin benzoate).
- Long-term solutions include: (i) establishing a monitoring and early warning system (between Vietnam and other neighbouring countries), (ii) applying information technology to forecast via remote sensing, (iii) applying IPM to maize production including use of tolerant varieties, and use of natural enemies such as parasitoids, earwig, other natural enemies.

Successful management of FAW depended on: (i) timely management directives that were well-coordinated and relied on both an efficient plant protection system and the national extension system, (ii) understanding the biology of the pest to enable effective control measures to be carried out across diverse ecological regions, (iii) provided training sessions to extension officers and farmers, and (iv) through collaborations with international organisations including FAO, CAAS, CSIRO, ASEAN FAW Action Plan, and the Crawford fund.

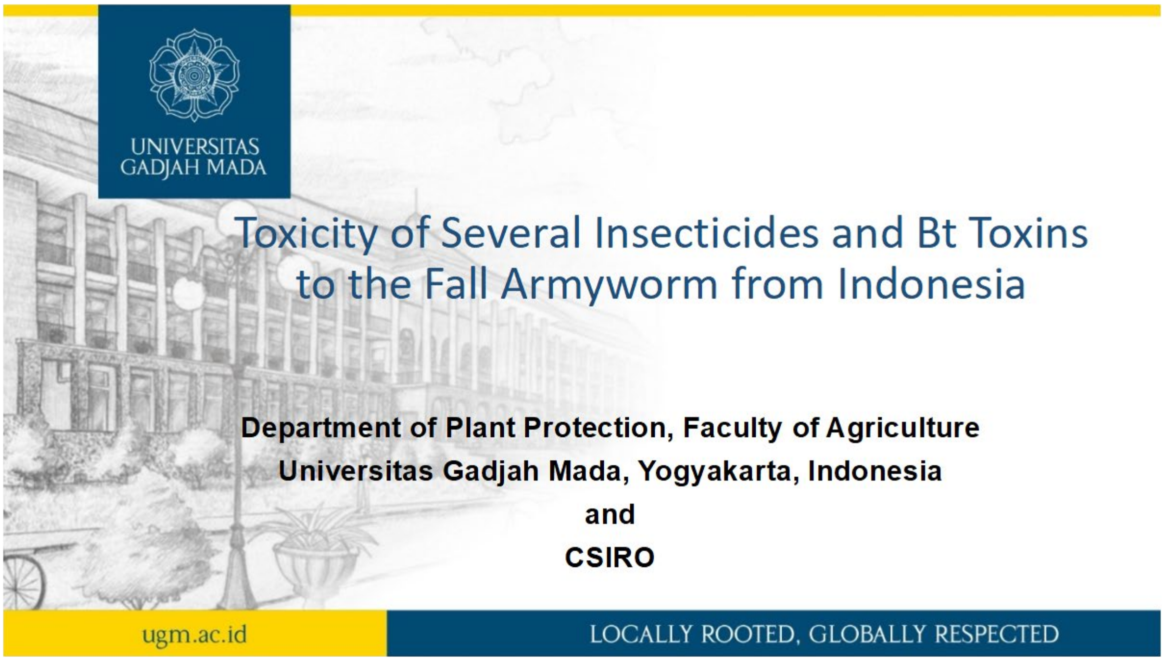
At the national level, research activities on the FAW included (i) studying the morphology / biotype of FAW, (ii) its biological and ecological characteristics, (iii) evaluating pesticides' efficacies on FAW and action threshold, (iv) use of pheromones and attractants to manage FAW, (v) evaluating susceptibility of different maize cultivars to the FAW, (vi) characterisation of natural enemies of FAW, (vii) understanding the pest's plant host range, and (viii) developing appropriate control methods such as cultural practices, biological control and chemical control options.

Trials of 'food spray' (i.e., using rice flour or yeast as food sources to attract natural enemies) were conducted on maize crops that showed reduced FAW infestation levels, increase natural enemies, and increase crop yields, potentially indicating natural enemies benefited from the additional food sources.

Future work in Vietnam would include (i) understanding pest's ecological characteristics (i.e., host range, migration, dispersal and movements, impact from natural enemies), (ii) monitor and forecasting using information technologies, (iii) understanding threshold level, (iv) apply biological control (e.g., mass rearing of natural enemies, develop and apply biopesticides), (v) training of use of biological control for farmers and extension officers, and (vi) application of IPM through continuous supply and availability of IPM tools.

6. Indonesia: Valentina Aryuwandari, Y. Andi Trisyono (Universitas Gadjah Mada)

Presentation Title: Toxicity of several insecticides and Bt toxins to the fall armyworm from Indonesia



SUMMARY

Heavy outbreaks of FAW in Indonesia was first reported in the newspaper Tribun-Medan on 1st May 2019 in the Karo District of the North Sumatra Province. Crop with similar damage symptoms in other regions including in the East Lampung District from Lampung Province was also observed on 15th May 2019 (Trisyono et al. 2019). In Indonesia, FAW damage was reported from 31,856 ha from 23/34 provinces in 2019, and 113,143 ha in 2020 from 28/34 provinces. Repeated insecticide spray on maize from South Lampung have not prevented severe damage of the maize plants, highlighting farmers' knowledge gap with respect to management of this invasive pest. Role of egg parasitoids (*Telenomus* sp, *Trichogramma* sp.) in managing the FAW has been investigated in agroforestry, rice fields, and rainfed field agroecosystems involving maize crops of different age by Wahyuningsih et al. (2022)

- The COVID-19 pandemic significantly impacted laboratory activities such as restriction on the number of people allowed to work in the laboratory concurrently
- Difficulty with rearing sufficient number of larvae at the appropriate developmental stages as required for specific insecticide bioassay experiments
- Genomic analysis of Indonesian FAW was not successfully carried out due to transportation difficulties as well as due to local legislations relating to sharing of biological specimens
- The need to ensure that all SEA project partners were using the same bioassay protocols to ensure meaningful comparisons of insecticide resistance profiles was possible
- Prioritising the enhancement of ecosystem services is needed for managing the FAW
- Insecticides and Bt toxins are alternative control strategies to keep the pest population low, but there is a need to improve farmers' knowledge on efficient application of insecticides to minimise risks including health risks, damage to ecosystems, and development of resistance in target pest

7. Uganda: Michael Hilary Otim (National Agricultral Research Organization)

Presentation Title: Update on fall armyworm research and management in Uganda

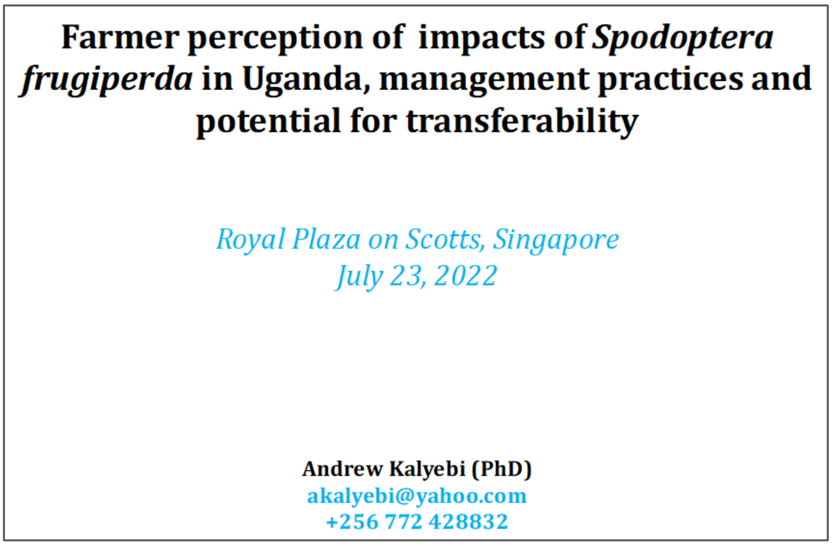
SUMMARY

FAW damage symptoms on maize first observed in May/June 2016, although farmers reported first seeing FAW as early as in 2014 and 2015. National task force on FAW documented spread extent and its socio-economic impact, with up to 50% grain losses attributed to FAW. In Uganda, molecular characterisation of FAW strain identity (Otim et al. 2018) and resistance alleles (Guan et al. 2021), and molecular diagnostics of parasitoids targeting FAW (Otim et al. 2021) have been undertaken.

- surveys indicated that most farmers attempted to manage the FAW using either chemical or cultural approaches in Uganda
- FAW control strategies recorded include: (i) insecticide spraying (90-96% of farmers surveyed); while other minor strategies included also applying: (ii) ash; (iii) cow manure; (iv) paraffin, (iv) pepper, (v) acaricide, (vi) using hand picking, (vii) plant trap plants.
- Mortality of 3rd instar FAW larvae by entomopathogenic fungi documented but fungal species unknown; efficacies under field conditions untested and will require further research. Mortality rates of different fungal isolates in laboratory/screenhouse evaluation ranged from >10% to >50%.
- FAW management in Uganda focused areas included: (i) surveillance to establish its status, (ii) carry out capacity building for FAW management, (iii) increase pest awareness, (iv) provide demonstration material to local government, (v) support activities of national task force, and (vi) research for sustainable management.
- Gaps identified included: (i) lack of decision tool for FAW control, (ii) limited knowledge on the bio-ecology and management of FAW, (iii) limited availability of IPM techniques including biocontrol, varieties, biotech crops, (iv) no cost-benefit analyses of different control options, (v) limited sharing of information among key players, (vi) weak institutional and infrastructural capacity (i.e., surveillance, monitoring, reporting), (vii) low private sector investment in research and business opportunities for FAW, (viii) ineffective central coordination efforts (quality control and synergism), (ix) lack of empirical data on socioeconomic impact of pesticide used to manage FAW.

8. Uganda: Andrew Kalyebi

Presentation title: Farmer perception of impacts of *Spodoptera frugiperda* in Uganda, management practices and potential for transferability



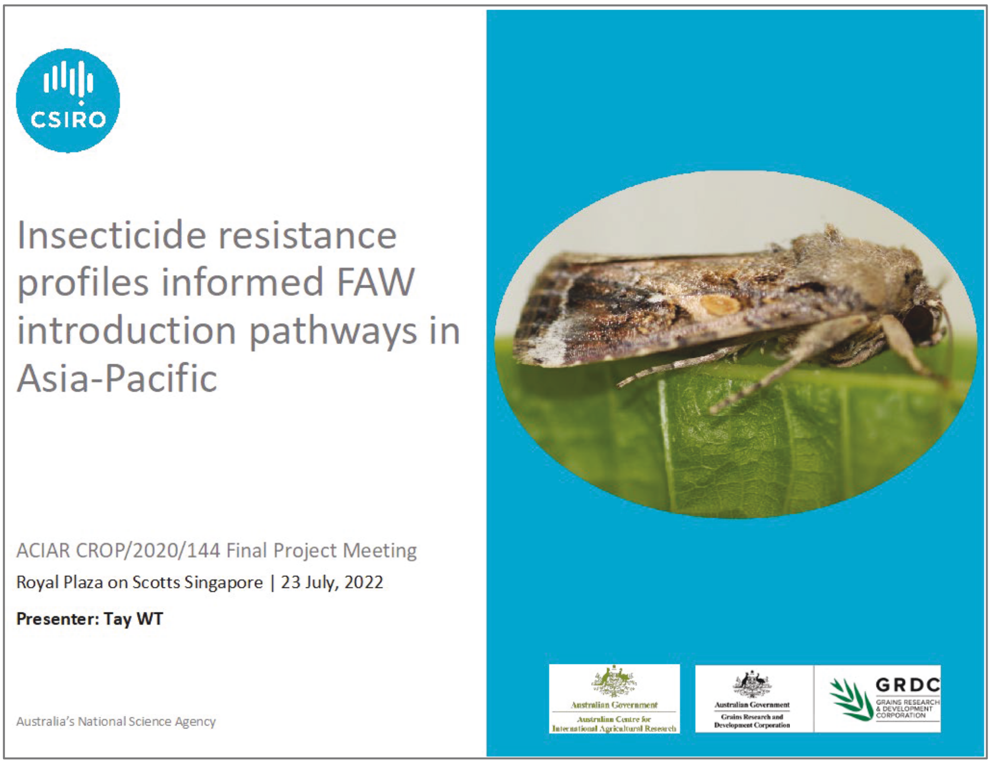
SUMMARY

Farmers in Namutumba district reported noticing FAW damage symptoms in their fields since 2013, while in Kamuli District farmers first noticed damage symptoms in 2014. >20% of farmers in Kamuli and ~10% of farmers in Namutumba noticed FAW damage symptoms since 2015. At the time of the field surveys in 2020 there were still farmers unaware of FAW in Uganda. FAW caused an estimated annual loss to 36% of maize production in Africa (economic loss = US$ 200 mil) by 2020. Different approaches to manage FAW being implemented by Ugandan farmers, shifting from the initial use of broad-spectrum insecticides that provided short-term reduction and control of pest population. There is an awareness for the need to integrate other non-chemical practices for sustainable management of the FAW, including cultural, mechanical, physical, and biological control options.

- Questionnaire surveys carried out in Kamuli and Namutumba Districts (Kalyebi 2021; Kalaybe et al. 2022)
- Between 50-60% of farmers surveyed from both districts reported economic impact from yield loss that ranged between 25-50%, while approximately 20-30% of farmers from both districts reported yield loss of >50%-100% yield loss due to FAW.
- >60% and >80% of farmers from Kamuli and Namutumba, respectively, perceived FAW as a very serious pest in their regions.
- 84% farmers from Kamuli and 90% farmers from Namutumba Districts reported to use chemical insecticides as an attempt to manage FAW but with varying degrees of successes and failures.
- 42% (Kamuli) and 44% (Namutumba) farmers also managed FAW by cultural practice of regular weeding.
- ~4% farmers did not take active actions against FAW due to poverty, cost of insecticides, and overwhelmed by the devastating effect of the FAW leading to abandoning the maize fields.
- Cultural and biological methods of FAW management included: (i) hand picking, (ii) early planting, (iii) use of organic manure as fertiliser, (iv) use of push-pull technique, (v) use of animal product such as animal/human urine, (vi) use of plant extracts including *Aloe vera*, tobacco, chili pepper, *Lantana camara*, and Neem (in combination with ash as catalyst) with farmers reporting 50-95% efficacy levels. Farmers considered these cultural and biological methods as effective but limited only to small sized gardens due to difficulties in ascertaining correct quantities to use.
- There is a need to redesign agroecosystem to both increase ecosystem services functionality and to reduce vulnerability to pests, reliance on pesticides and enhance biological control.

9. Australia: Wee Tek Tay (CSIRO)

Presentation Title: Insecticide resistance profiles informed FAW introduction pathways in Asia-Pacific



SUMMARY

• Population genomic studies based on 890 neutral and unlinked genome-wide SNP markers did not support a 'west-to-east' spread from West Africa to Asia for the invasive FAW populations. This study was published in Communications Biology (2022, 5: 297), led by CSIRO Health & Biosecurity (Tay, Rane, Gordon et al.) and involved partners from Uganda (NaCRRI), Brazil (UFG), France (INRAE), UK (Cambridge University), and China (NJAU).

• Genome-wide SNP markers identified Asian FAW populations was instead migrating to East Africa (Malawi, Uganda), but a knowledge gap remained for the invasive FAW populations in Southeast Asia and Australia.

• Bioassay experiments involved two FAW populations from Queensland (colony code Sf20-1) and Western Australia (colony code Sf20-4), tested via: (i) diet incorporation (for chlorantraniliprole, indoxacarb, Emamectin benzoate, spinetoram; on 2nd/3rd instar larvae), (ii) topical application (for alpha cypermethrin, methomyl; on late 3rd/early 4th instar larvae, each weighing ~30mg), (iii) surface treatment (for Cry1Ac, Cry2Ab, Cry1F, Vip3A, Xentari (Cry1D, Cry1C, Cry1Ac, Cry1Aa), and Dipel (Cry2A, Cry2B, Cry1Ab, Cry1Aa, Cry1Ac); on neonates). Initial findings were reported on GRDC Fall Armyworm portal https://grdc.com.au/resources-and-publications/resources/fall-armyworm; full results from Tay et al. (2022b).

• Australia FAW Bioassay data were co-analysed with whole genome data of invasive populations from Australia, PNG, South Korea, and native population from Peru.

• Toxicity ratio of FAW responses to various Bt and VIP3A toxins, and to insecticidal chemicals were assessed against *Helicoverpa armigera* where appropriate. This enables meaningful interpretation on how farmers can better manage this new invasive pest as measured against existing RMP relating to *H. amigera.*

• Literature reviews to identified results from comparable bioassay protocols were also undertaken, this enabled meaningful comparisons of how different invasive and native populations responded to selected insecticides.

• Significant resistance to methomyl was found in South African FAW as reported by Eriksson (2018)

• Significant resistances were observed in Sf20-4 population from Western Australia for Chlorantraniliprole (diamide) and for indoxacarb (organophosphate) as compared with the Queensland FAW Sf20-1. Profiles of responses from SEA FAW populations were unknown, however the results suggested independent introductions of FAW into Australia. Rapid development of responses to these insecticides were potentially unlikely when taking into consideration the findings by Kulye et al. (2021) involving multi-year studies of Indian FAW populations

• Analysis of the ACE-1 allele frequencies from the current project teams from Australia and SEA, and incorporating various published studies, identified patterns of resistance alleles that were inconsistent to the west-to-east spread for this pest.

• Taken as a whole, the bioassay and genome analysis suggested Australian populations: (i) have different resistance profiles, (ii) no Bt/VIP3A resistance phenotypes detected in Australia; (iii) invasive FAW populations have unique insecticide resistance profiles; (iv) resistance and bioassay results supported multiple introduction pathways to norther Australia regions. The results also provided cautionary consideration for the need to ‘get the story right’, and the need to now consider the role of climate change on pest adaptation and how they impact on regional/global farming communities.

10. Australia: Rane Rahul (CSIRO)

Presentation Title: Genetic Signatures informed FAW movements in APAC

SUMMARY

• Genomic markers were used to study the population dynamics and gene flow between the 36 invasive populations across 13 countries and 9 native populations across 7 countries.

• Complex introductions of Spodoptera frugiperda (FAW) were identified in Asia and South-East Asia.

• Most significantly, 6 clusters were identified in China and 3 clusters in Malaysia, further demonstrating multiple introductions of FAW.

• Gene-flow analysis also indicated uni-directional flow into Africa from Asia and bi-directional flow within Asia, with the oldest incursions in Asia recorded back in 2008 in Vietnam

• Australian populations in the 'east' found to be distinct to the 'western' populations.

• Phylogenetic trees further suggested multiple introductions into Australia since they don't cluster in one branch, but are instead found to be similar to multiple Asian populations.

• This also contradicts the theory postulating 'wind dispersal' from Asia into Australia.

• Signatures of differentiation identified within the Australian populations, suggesting the existence of a selection pressure.

• 'East-to-west' spread more likely than 'west-to-east'

• These work highlight the limitations of 'marker-based' studies, which cannot assess fast-evolving population dynamics.

• Future work will involve data generation in Australian and New Zealand populations, genome wide association studies to identify resistance alleles, forward simulations to assess the impact of genetic drift on inter-population variation and genomic architecture assessment to identify any gene expansions that may be related to increased spread.

• Finally, these analyses will inform future research into climatic adaptation associated genetic structure and expansion of invasive pest ranges.

10. Singapore: Dr Alison Watson (ASEAN FAW ACTION PLAN)

Presentation Title: Update on ASEAN FAW Action Plan CSIRO Workshop 23 July 2022

SUMMARY

• The ASEAN FAW Action Plan provides a unique regional 10 nations response to the challenge posed by the FAW, and was signed off at Ministerial level as an ASEAN regional strategy, and serves as a model for emerging pests and diseases.

• The regional response is needed to tackle a fast moving transboundary pest, ASEAN farmers urgently need effective, locally validated and regionally relevant management solution. It also promotes the sharing of information and resources, and help to build trust between private and public stakeholders and farmers to drive long term Change.

• Three goals: Reduce FAW/pest-induced crop losses and impact on livelihood; Promote. Sustainable and cost-effective IPM, and Drive coordinated and effective multi-stakeholder communication.

• Six objectives: (i) Support national capacity-building, (ii) executing an ASEAN research development and technology implementation agenda, (iii) promote information transfer and adaptive learning, (iv) consolidate critical knowledge base, (v) Establish ASEAN-wide cost effective pest intelligence network, (vi) mobilizing resources.

• Seven thematic work programs: (i) knowledge innovation hub, (ii) monitoring/surveillance, (iii) farmer communication, (iv) resistance, (v) biocontrol, (vi) Drones and digital IPM, (vii) women as IPM leaders

•Regional resistance management plan: (i) regional FAW surveillance and resistance, (ii) country-specific resistance management guidelines, (iii) integrating host plant resistance with other compatible IPM tactics for sustainable FAW control.

- Regional discussion on connections, collaboration and priorities: Part 1 – Climate change and transboundary plant pests and diseases in SEA (focus on FAW), Part 2 – The role of genomics in understanding strategies for management of plant pests and diseases in SEA (focus on FAW)

• Women as IPM leaders concentrating on communication/information & knowledge, enabling environment, leadership/entrepreneurship, education/training, mainstreaming

• Biocontrol program involving knowledge sharing, regulation, research capability (DFAT-funded ABRC), pilots demonstration/technology transfer (ASEAN Biocontrol Accelerator), and farmer education and training

• Farmer toolkit pilot in Vietnam to showcase FAW control strategies

• Drones and digital IPM – develop concept paper to build confidence in drone IPM in smallholder farmers, support digital agricultural technologies, and promote future development and integration of drone and digital solutions to improve resource, profitability, transparency and sustainability.

10. Singapore: Amelia Fyfield (CSIRO Business Development & Global)

Presentation Title: An ASEAN Bio-Protection Research Centre

SUMMARY

• ASEAN member countries facing complex transboundary agricultural pest threats, including the African Swine Fever and FAW that are spreading across SE Asia and into the Pacific, and will require a strong and effective regional collaboration on biosecurity to address these threats. These caused widespread economic losses and food insecurity issues which are of concerns to all partner countries; globalisation driving spread of these diseases.

• Need a coordinated multi-country research and actions is essential to better understand monitoring and prevent impact of invasive agricultural species (IAS); and the need of an ASEAN Bioprotection Research Centre (ABRC) was conceptualised

• The ASEAN FAW taskforce recommended this to the secretariate of the ASEAN FAW Action Plan, and working in collaboration with CSIRO to run a co-design process for an ABRC.

• The idea of being at the end of an extensive co-design process to come up with a blueprint on how the centre might operate, the kind of funding it might draw in, and the kinds of work the centre might do, i.e., looking at how to collectively prioritise work to do across the region.

• The centre will help build ASEAN capability and capacity around the introduction, scaling up, and use of biprotection technology and approaches for agricultural pests and diseases in the region.

• CSIRO is now launching this comprehensive ideation and co-design processes with regional stake holders and now in the process of going to the market to identify the consultancy firm to support this comprehensive and thorough consultative process.

• Through this meeting, CSIRO will seek input from this research team to find out who we should engage in this ABRC co-design process from the ASEAN region, working through 4 phases: **[i]** July 2022: explore what is desirable (conduct 20+ one-on-one interviews with ASEAN stakeholders), **[ii]** Aug/Sept 2022: frame what is possible (conduct 6-8 'focus groups' design sessions with key stakeholder cohorts to further explore what we should be focusing on, who is best placed for the various research tasks); **[iii]** Oct 2022: Design what is viable (facilitate a symposium bringing together diverse participants to identify priority key research gaps and opportunities for collaboration); [iv] Nov/Dec 2022: Communicate with key stakeholders what is scalable (validate and deliver a blueprint for an ASEAN ABRC) – defining what the challenges are, how the model will operate, how it will be funded, and how it can move forward.

• Stake holder mapping session: To showcase key players and stakeholder cohorts in the ASEAN Bioprotection ecosystem. It will be built-upon through conversations with the project team, interviews with key stakeholders and desktop research.

• Using the mapping session to also highlight the anticipated levels of engagement these individuals and groups will experience with The Hub. The dotted rings represent the frequency of input, with the Bio-Protection Experts being heavily engaged throughout the design process.