FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

UM00049

Management of insecticide resistance in RLEM and screening new MoA chemistry

PROJECT DETAILS

PROJECT CODE:	UM00049
PROJECT TITLE:	MANAGEMENT OF INSECTICIDE RESISTANCE IN RLEM AND SCREENING NEW MOA CHEMISTRY
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Summary

Redlegged earth mite (RLEM) is a significant threat to the establishment of grain crops. This research has led to recommendations about insecticide resistance management and improved chemical control methods for RLEM. Field surveillance, population genetics, fitness studies, and predictive models were integrated to guide long term management and monitoring across southern Australia. Notably, these efforts revealed that synthetic pyrethroid# (SP) resistance is widespread across Western Australia (WA), and discovered the first populations resistant to organophosphates# (OPs). This project has helped accelerate the registration process for a new chemical product for mite control in grain crops.

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Conclusions

Surveillance of hundreds of RLEM populations across Australia has found that SP# resistance is widespread across WA, but currently not found in any other state. Examination of paddock histories and simulation studies suggests that differences in climatic factors, as well as chemical practices, contribute to these observed differences in resistance across Australia. The mechanism of SP resistance was found to consist of a novel kdr mutation in the parasodium channel gene, which was used to develop a cheap, fast and reliable method for the detection of resistance. Genetic work suggests long distance spread of resistant mites has occurred, and also revealed SP resistance has evolved on multiple occasions in WA. While passive long distance dispersal of RLEM may be difficult to control, the independent evolution events highlight the importance of implementing strategies that minimise selection for resistance. This may be particularly important for RLEM in light of findings that suggest the fitness costs of SP resistance appear to be relatively small.

A resistance management strategy was developed by the National Insecticide Resistance Management (NIRM) working group using knowledge acquired under this project and tools for simulating the effect of different scenarios on the evolution of resistance. These strategies include the rotation of chemicals, minimising chemical applications through use of non-chemical control methods, avoiding weak chemical exposures, reducing weeds, and paying attention to fence lines and other RLEM refuges. The discovery of the first populations of RLEM resistant to OPs# highlights the need to improve pest management practices and the importance of ongoing resistance surveillance to be coordinated across Australia. Adoption of these resistance management strategies has been assisted by the large extension efforts throughout the project, and will benefit from further support of professional service providers with skills in resistance management and integrated pest management (IPM) decision making.

Recommendations

The RLEM is a significant threat to the establishment of grain crops and has evolved resistance to SPs# and, more recently, OPs#. As these two chemical classes are the only two foliar controls currently registered for RLEM, the emerging resistance to OPs will need to be characterised more carefully so that it can be properly managed. A new GRDC funded project (UM00057) will extend many of the research areas surrounding resistance. This includes characterising of the genetic basis of OP resistance, continuation of a resistance surveillance program, and developing laboratory techniques to determine chemical sensitivity data for other chemicals (e.g. neonicotinoids#), as well as other important pests (lucerne flea and bryobia mites).

However, there still remain key research areas that are not being investigated and warrant consideration:
1. RLEM monitoring tools for growers (including exploration of automated traps and models that improve local level information of RLEM numbers).
2. Development of IPM-based strategies for RLEM that include biological and cultural control methods, and that fit into modern broadacre systems (particularly for regions where high levels of resistance have been found).
3. The development of dynamic and robust economic thresholds for the chemical control of RLEM.
4. A more effective means to assess the risk of RLEM damage to susceptible crops through the development of a pest severity

index using local agronomic, pest and climatic information.
5. Understanding whether genetically distinct RLEM populations are more or less likely to evolve insecticide resistance, linked to underlying frequency of rare resistance alleles.

Outcomes

The results from this project have significantly increased the previously sparse knowledge surrounding resistance in RLEM. This knowledge, as well as tools developed in this project, have enhanced the ability to respond to and manage this emerging threat to Australia's grains industry.

Notably, 1) A cheap and fast method for the detection of resistance to pyrethroid# insecticides has been developed, 2) Extensive surveillance has been undertaken to map the spatial extent and level of resistance, which led to the discovery of the first RLEM population resistant to OPs#, 3) How resistance may spread or evolve has been quantified, 4) The mechanism conferring pyrethroid resistance to RLEM and some of the associated fitness costs have been uncovered, 5) Modelling tools to predict how the risk of resistance may vary for different contexts such as locality, climate, land usage, chemical usage, have been developed, 6) A number of potentially 'new' chemicals have been tested for RLEM control, and data packages for a new insecticide mode of action (MoA) have been identified and subsequently developed, and 7) These findings have been widely disseminated to stakeholders, mostly growers, advisers and agrochemical companies, in order to help them identify and manage resistance. The data were integral to the development of a resistance management strategy for RLEM commissioned by GRDC and produced by the NIRM working group in 2015-2016.

This project has provided an increased understanding of current resistance management risks, allowing growers and advisers to manage RLEM and insecticide resistance in the most appropriate manner. Adoption of resistance management practices by growers will reduce fuel and input costs, and minimise chemical control failures and the need to 're-spray' paddocks multiple times. This will reduce the chemical load on the environment, particularly associated with broad-spectrum pesticides which are known to have negative impacts on soil flora and fauna, beneficial insects and water quality. Coupled with these smarter practices, the likely registration of a new MoA, that was made possible through this project, will help to maintain the viability of control options and ensure yield losses due to RLEM feeding damage are minimised into the future.

Achievements/Benefits

Background
Pesticides are an effective and widely used pest control method, but emerging resistance issues have necessitated a rethink of management approaches. Pest control failures due to chemical resistance reduce quality and yield expectations of growers, and increase input costs due to the greater amounts of chemicals required to achieve a given level of control. The current trajectory of pesticide use suggests resistance issues will almost certainly continue to increase.

Recently, resistance issues have emerged for one of the most important pest species to the Australia's grains and pastoral industries, RLEM, (*Halotydeus destructor*). RLEM continues to be an intractable pest causing damage to pastures and most oilseed, cereal and pulse crops in southern Australia. In 2006, chemical control failures were reported from a population of RLEM in WA and later confirmed as the first documented case of insecticide resistance in this species. Very high levels of resistance were found to multiple SPs#, but not to other chemical classes, including OPs#, carbamates#, phenylpyrazoles#, avermectins# and diafenthiuron#.

Prior to commencing this project, major gaps were identified in the knowledge and ability to respond to resistance in RLEM. The gaps to be addressed during the course of this project included 1) A lack of cheap, and fast molecular methods for the detection of resistance, 2) A lack of knowledge surrounding the spatial extent and level of resistance, 3) A poor understanding of how quickly resistance may spread or evolve, 4) A lack of knowledge surrounding the mechanism conferring resistance and any associated fitness costs, 5) An inability to predict how the risk of resistance may vary for different contexts such as different locations, climates, land usages and chemical usages, 6) An over-reliance on a small number of chemicals for the control of RLEM, and 7) A lack of information outlets to encourage broader understanding of resistance issues in RLEM among growers.

Through an integrated approach between The University of Melbourne (UM), CSIRO, the Department of Agriculture and Food WA (DAFWA), CESAR and the University of WA (UWA), this project has successfully addressed these identified gaps. This has led to recommendations about insecticide resistance management and improved control methods for RLEM.

Major achievements
1. Development of a diagnostic for resistance and delivery of a testing service
A cost effective method was developed to rapidly screen mite populations for SP resistance. The method is highly sensitive and can be used to detect even small numbers of resistant individuals in large samples (e.g. more than 1,000 individuals). Early in the project, a putative mechanism for SP resistance was identified using a whole genome sequencing approach, and eventually confirmed through mites collected from WA and Victoria (VIC) treated with various doses of SPs in bioassays. This work formed the basis for a genetic diagnostic. The approach was validated extensively and used as an important part of this project's resistance monitoring program.

2. Insecticide resistance surveillance across southern Australia
The extent of SP resistance across WA and eastern Australia (South Australia (SA), VIC, New South Wales (NSW) and Tasmania (TAS)) was monitored and mapped through a broad scale surveillance program. Across the 2014-2015 period, mites were collected from 250 populations in WA and 131 populations in eastern Australia (each with 5-year paddock records and spray histories). These populations were screened for SP and OP resistance using both molecular and bioassay approaches. As part of this effort, 26 new populations of SP resistance were identified in WA and are now being managed. In addition, this monitoring program revealed the first population of RLEM known to be resistant to OPs. Further OP resistant populations were identified throughout the course of this project. This finding is significant as there are now no remaining chemical groups registered for foliar control of RLEM effective against populations resistant to SPs and OPs.

3. Studies to understand movement patterns and resistance evolution
Despite SP resistance being detected prior to the commencement of this project, the biological mechanism responsible for resistance and how it would continue to evolve and spread was still unknown. This project developed robust field sampling methodologies and mapped the spread of resistance at both smaller scales (e.g. between paddocks, along fence lines) and larger scales (more than 100km). Here it was revealed that resistance is relatively slow to spread between paddocks, due to the limited dispersal capacity of mites, and the spread between paddocks occurs mostly along fence lines. Resistant mites may also disperse passively via wind or farm equipment, and this type of long distance spread of resistance was detected in one population through genetic analyses.

The project also developed ddRADseq markers (high density reduced representation markers) for RLEM. This has provided several thousand high quality single nucleotide polymorphisms (SNPs) across the genome for detailed genetic analysis. This has been used to assess local movement patterns and confirm the occurrence of independent evolution events. In addition, the data show eastern and western populations of RLEM can be genetically distinguished. Building this understanding was important for developing effective resistance management strategies.

4. Data packages for new MoAs against RLEM
This project's discovery of OP resistance in RLEM has emphasised the need for alternative MoA chemicals for which resistance does not exist. Discussions were held with all major agrochemical companies (and many smaller companies) to identify possible candidates. Laboratory bioassays and two microcosm trials were then completed on 12 candidate products (with comparison to conventional 'control' insecticides). This included many new MoAs for RLEM. From these trials, and after discussion with participating companies about market potential, five actives were chosen for testing in the field. In 2014, these were compared to omethoate[#] and alpha-cypermethrin[#] on pastures and canola, in both WA and VIC. Results from the field trials showed one MoA is highly efficacious against RLEM. Two further products showed some efficacy on pasture only, while the remaining two products failed to perform under field conditions. In 2015, an additional set of field trials (three in WA and one in SA) was undertaken. In these trials, the efficacy of the most promising product was evaluated at several rates on canola at the cotyledon stage and again at the second true leaf stage. The product was compared with two commercially available insecticides, omethoate and alpha-cypermethrin, and an untreated control. Pest numbers, crop damage, plant counts, crop vigour and phytotoxicity were assessed prior to spraying and again approximately 3, 7, 14 and 28 days after treatment (DAT). These trials confirmed product efficacy and have yielded valuable data packages for the relevant agrochemical company that will be integral to the registration of this compound. Residue trials for this chemical are now underway, and research is simultaneously being undertaken on aphids and diamondback moth (DBM) pests that are also prone to insecticide resistance.

5. Understanding of OP resistance in RLEM

To better understand the mechanism conferring the newly discovered OP resistance, a combination of genetic and chemical bioassay studies was performed. These studies were used to determine whether resistance was conferred by a target site modification or through metabolic detoxification, which has implications for how the resistance may persist and evolve, and thus, how it should best be managed. Using laboratory bioassays, it was shown that resistance exists across multiple OP chemicals, including omethoate, chlorpyrifos# and malathion#. Chemicals that selectively synergise the activities of insecticides against P450 monooxidases, esterases, and glutathione-S-transferases detoxification mechanisms were used to determine whether any of these mechanisms are contributing to OP resistance in RLEM. None of the three synergists completely removed the increased survival observed in the resistant population relative to the susceptible population, although there was some evidence to suggest that esterase metabolic activity could be contributing to the resistance.

The acetylcholinesterase (AChE) gene in RLEM was characterised by using a combination of genomic tools. The next steps are to sequence a region of AChE in mites from several susceptible populations from WA and VIC, as well as the resistant populations from WA. Presence of a non-synonymous mutation(s) in the resistant population, but not the susceptible population, will likely indicate target site resistance in the AChE gene for OP resistance.

6. Modelling resistance
In order to predict the spread and evolution of resistance under different contexts (e.g. new locations, climates, land usages, chemical usages, etc.) and into the future, modelling approaches were developed that simulate biologically realistic mite populations under various conditions. This allowed different potential resistance management strategies to be assessed by predicting their effect on the spread and evolution of resistance at various spatial scales. It also allowed the prediction of how resistance was likely to develop into the future without any intervention. Results confirmed that untreated edges, such as fence lines or near trees, deserve particular management attention as they may serve as migration corridors for mites. Other findings showed a strong climatic signal in the distribution of SP resistance in RLEM, with aridity, temperature seasonally, and precipitation patterns constituting strong predictors of resistance. This information was used when the NIRM group developed the first resistance management strategy for RLEM.

7. Communication and extension activities
There has been a large focus on communication and extension across this project in order to rapidly translate new findings to growers that will help better manage resistance in RLEM and minimise yield losses. Numerous industry presentations have been given in WA, VIC, SA and NSW to increase awareness of insecticide resistance in RLEM and enlist growers in identifying chemical failures. Media releases have been produced through the GRDC and press articles published in various forums targeting growers and advisers, including Ground Cover, Paddock Practices and the Stock Journal. Research results have also been presented internationally and have led to a number of scientific publications (two published, two submitted and four in preparation). In total, there have been almost 150 extension activities delivered throughout the project. Project personnel and scientific findings were crucial to informing a resistance management strategy developed through the NIRM working group. This resistance management strategy will be publicised prior to the 2017 growing season.

Benefits to industry
The discovery of resistance to OP and spread of SP resistance in WA highlights the importance of resistance surveillance of RLEM populations. This project has optimised the process of screening for resistance, which can now be undertaken quickly, and at a relatively low cost. This has strengthened the Australian grains industry's ability to track and respond to resistance issues in RLEM. Enhanced knowledge of genetic and spatial aspects of resistance has fed into predictive tools developed in the project that allow the projection of current trends so that future scenarios can be planned for before they occur. This is particularly important in a resistance context, as early detection and intervention of resistance increases the likelihood that resistant populations will revert to susceptible, and ensures the viability of control measures into the future. More generally, this project has provided a scientific basis for regionally specific resistance management strategies in Australian crops and pastures as part of the GRDC commissioned RLEM resistance management strategy. These strategies provide recommendations for the optimal use (and rotation) of insecticides, while combining non-chemical options such as targeted weed control, host plant resistance and crop rotations. The increasing presence of both SP and OP resistance discovered throughout this project highlights how important it is that these strategies are implemented by Australian growers. Extensive communications to stakeholders throughout the project have been vital to the adoption of such management practices.

New chemical active ingredients for RLEM are important for the long term management of resistance, however there are very few genuine alternatives that are available to the grains industry. From a control perspective, the presence of mite populations with dual insecticide resistances is particularly concerning. Once a crop has been sown, there are no chemical options available in those paddocks where mites possess resistance to both OPs and SPs. In combination with providing better strategies to minimise the evolution of resistance, this project has provided a platform to accelerate the registration process for new insecticide products for mite control. In particular, data packages have been generated and these have been provided to one agrochemical company for a new MoA chemical, which will be submitted for registration in the next 12-18 months.

Other research

Many of the techniques and expertise developed in this project are relevant to other pests in the grains industry, as well as other chemicals used for their control. Such extensions are being pursued in a new GRDC project (UM00057) that, among other objectives, will generate chemical sensitivity data for other grains pests such as lucerne flea and bryobia mites, as well as other chemicals, such as the class of neonicotinoids[#].

Intellectual property summary

Trial data from the screening of new MoA chemistries will not be made publicly available.This data will be used to assist registration of a new insecticide product in grains. All other research outputs from project UM00049 are intended for the public domain, consequently no intellectual property (IP) is envisaged.

Additional information

Attachments

1. Experimental and Applied Acarology article.

2. Resistance maps showing distribution of RLEM.

3. Biodiversity Research article.

4. Activity and extension list.

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