2020 FLYSTRIKE PREVENTION RD&E PROGRAM

PROJECT SUMMARY REPORTS

LETTER FROM THE AWI CEO

AWI is determined to ensure that woolgrowers have access to best practice 'welfare-improved' flystrike prevention, backed by science, that increases the demand for Australian wool.

On behalf of Australian woolgrowers, AWI runs a robust flystrike research, development, education, extension and communication (RDEEC) program aimed at ensuring the lifetime welfare of individual sheep whilst reducing the industry's reliance on mulesing. Our objective is to provide woolgrowers with a range of practical options to prevent flystrike, whatever their sheep type, environment or business priorities.

Every two years since 2008, AWI holds a special one-day technical forum for key producer representatives, animal welfare representatives and researchers to receive updates and provide feedback on this flystrike RD&E program. Attendees also hear of the progress being made by woolgrowers to adopt best practice flystrike management. The forum is regularly attended by more than 100 woolgrowers, researchers, industry consultants, commercial providers, veterinarians and animal welfare advocacy groups.

Unfortunately, the 2020 forum had to be cancelled, due to COVID-19 public health guidelines. However, I am pleased to present this compendium of summary reports of current and recently completed AWI projects provided by the researchers.

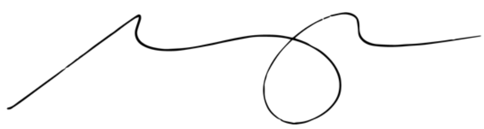
AWI's flystrike program is achieving significant and incremental progress across a wide range of research projects and trials conducted on farms and in laboratories. The program is a key investment focus of the current AWI strategic plan, with targets that include:

- Evidence of successful development of a flystrike vaccine prototype.
- Evidence of investigations into novel pain relief options.
- Developed integrated parasite management strategies to minimise the impact of chemical resistance.
- Demonstrated 10% increase in adoption of welfare improved practices.

In the past, feedback and comments from attendees of the one-day forum have been very beneficial, with input contributing to new projects. Despite the cancellation of this year's forum, we still very much encourage feedback on these summary reports and any other aspect of our RD&E program. Please provide your feedback to AWI's Program Manager for Sheep Health & Welfare Bridget Peachey at email: bridget.peachey@wool.com.

The global coronavirus pandemic affects the sheep and wool industry in many ways, however most of our on-farm R&D projects continue as normal and we closely monitor their progress. AWI remains very much open for business.

I hope you have all been safe and well during these unprecedented times and remain so.



Stuart McCullough
June 2020

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University of Melbourne researchers have used CRISPR gene modification technology to knock out the eye colour gene of the sheep blowfly, producing a fly with white eyes (right). The technology will play an important part in determining the best genes in the blowfly to target for control of flystrike (see page 22).

AWI FLYSTRIKE PREVENTION RDE PROGRAM OVERVIEW

AUTHOR
Bridget Peachey, AWI Program Manager Sheep Health & Welfare

OVERVIEW
At the previous Flystrike RD&E Technical Update, held in July 2018, Dr Peter James presented on a collaborative AWI project that had recently commenced to review the risk factors for breech flystrike. This review (ON-00510) has since been completed and outcomes from the work are included in the project summary reports. In preparation for this review a one-day workshop, run by Ausvet Pty Ltd with parasitologists, entomologists, animal production experts, geneticists and research program leaders, was held to develop a causal web for breech flystrike.

The breech flystrike causal web has since been reviewed and enhanced and is now available electronically at www.wool.com/flystrikecausalweb. It highlights the complexities of the task Australian woolgrowers face with respect to managing this significant disease and the challenges involved in identifying solutions that meet the diverse range of sheep type, environment and farm business priorities under which they operate.

AWI has recently endorsed an updated *Flystrike Research, Development, Education, Extension and Communication Strategy 2019/20 to 2024/25,* to guide investment in evidence-based RD&E to ensure the lifetime welfare and productivity of sheep and reduce reliance on mulesing (see Appendix 1). This strategy update is largely a continuation of AWI's previous flystrike strategy, with industry continuing to support a balance between long- and short-term investment in flystrike prevention RD&E.

A key event on the AWI calendar, held since 2008, is a biennial one-day flystrike prevention RD&E technical forum for producer representatives, animal welfare representatives and researchers to receive updates and provide feedback on the program. Unfortunately, due to COVID-19 public health guidelines, the 2020 forum had to be cancelled. However, in the past two years, since the previous forum in 2018, the Flystrike Prevention RD&E Program has made significant progress. Some of the RD&E highlights since the last forum follow, and updates on these projects and others are presented in a collection of summary reports of current and recently completed AWI projects, provided by the researchers. These projects represent AWI's diversified investment in the principles of integrated pest management in the search for practical solutions for woolgrowers to prevent flystrike, ensuring the lifetime welfare of individual sheep, whilst reducing reliance on mulesing.

NON-INVASIVE MANAGEMENT PRACTICES
Non-invasive management tools are pivotal in reducing the risk of flystrike. These include the strategic timing of crutching and shearing, controlling dags and the judicious use of chemical treatments. Parasiticides for the prevention and treatment of all forms of flystrike and worms are important tools in managing flystrike risk and when used strategically and in conjunction with other management tools, further reduce the risk of flystrike in sheep. Better management of current chemicals, the improved delivery of these chemicals and the development of new chemicals will minimise the risk of worms and blowflies and their larvae developing chemical resistance, ensuring that growers retain access to effective chemical treatments into the future to improve the lifetime welfare of all sheep. The development of vaccines against the first stage larvae of the Australian sheep blowfly (*Lucilia cuprina*) to decrease the susceptibility of sheep and potentially the future development of soil biocides to reduce the adult fly population will add to the management options and reduce the reliance on chemical use and breech modification.

An update to the blowfly genome, completed in 2019, identified 572 genes that are unique to the blowfly and which could be targeted by new chemical treatments or vaccines. Outcomes from this project are already being used to identify potential flystrike vaccine candidate genes that might impact larval growth and development, in a collaboration between the University of Melbourne and CSIRO to develop a flystrike vaccine.

A review of sheep blowfly pathogen control (ON-00620), undertaken by AgResearch, identified a potential novel biocontrol agent *Tolypocladium cylindrosporum* (*T. cylindrosporum*), as capable of killing *L. cuprina*. Whilst there is no record of *T. cylindrosporum* being present in Australia, an initial review of sequencing from Australian soil samples by the researchers suggested that there is a possibility. A project, currently underway (ON-00721 Identifying the presence of *Tolypocladium cylindrosporum* in Australian sheep growing areas), is genetically screening samples of fresh soil, obtained as part of a current University of Melbourne project into *L. cuprina* populations (ON-00624) from several sheep properties in Victoria and Tasmania, using DNA isolation and sequencing to confirm if *T. cylindrosporum* is present.

A project, led by CSIRO (ON-00723 Fleece rot control by vaccination: feasibility review and options), is nearing completion. It will deliver a comprehensive literature review and report on the challenges and feasibility of developing a viable vaccine(s) for sheep targeting the known pathogenic bacterial species (*Pseudomonas spp.* (fleece rot), *Dermatophilus congolensis* (lumpy wool) and other bacterial species) that contribute to the incidence of flystrike.

An investigation into the levels of blowfly resistance to the available chemical treatments (ON-00491) will inform further RD&E supporting growers on the judicious use of parasiticides through an integrated pest management approach to maximise flystrike control and maintain the efficacy of available insecticides. The Sheep Blowfly Resistance Management Strategy Working Group have developed information for woolgrowers on the appropriate use of chemicals to prevent or reduce resistance on their properties. Its members are Brian Horton (University of Tasmania), Peter James (University of Queensland), Deborah Maxwell (ParaBoss), Nick Rolls (Elanco), Jane Morrison (Coopers Animal Health) and Narelle Sales (NSW Department of Primary Industries).

BREEDING AND SELECTION

Breeding sheep naturally resistant to all forms of flystrike is a long-term solution to managing the risk of flystrike. However, there is no one-size-fits-all sheep breeding program and breeding strategies need to be customised to the individual farm, guided by key principles identified from AWI's investment to date in breeding for breech flystrike resistance. Breeding strategies must also integrate objectives for other health and welfare traits such as conformation and reproductive performance. AWI invests strongly in identifying and promoting optimal breeding tools for growers to meet their breeding objective. Examples include Wether Trials, Ewe Trials, Australian Merino Sire Evaluation and MERINOSELECT.

Australian Sheep Breeding Values (ASBVs) for the flystrike indicator traits, breech wrinkle, breech cover and dag score, implemented since 2009, are now available for use by ram breeders and wool producers on 20-30% of recorded animals in MERINOSELECT. A 2019 modelling project to investigate the rate of genetic gain when breeding for flystrike resistance (ON-00524) found that genetic gains can be made in reducing susceptibility to flystrike whilst simultaneously making gains in the major production traits, including fleece weight and fibre diameter. Some ram breeders are utilising these findings and are increasingly breeding lower worm egg count, lower breech wrinkle animals with higher fleece weight, as illustrated in the MERINOSELECT genetic trends. Although modest, genetic trends for lower breech wrinkle are occurring, particularly in medium and fine/medium wool Merino types. There are individual breeders specifically breeding Merinos for the package of low wrinkle, low dag and high productivity and these breeders are making considerable gains. The gains are easiest in medium wool Merinos in low dag country but quite difficult in Fine and Super Fine Merinos in high dag country. Further information is provided in the project summary report, Breeding and Selection – Industry trends.

Whilst investigations to identify the DNA differences or genomic associations between highly resistant and susceptible sheep for breech flystrike (ON-00515) did not find any major genes associated with breech flystrike, results indicated that future breech trait ASBVs, enhanced with genomic markers for flystrike susceptibility will be an effective tool to further increase genetic progress in breech flystrike resistance. Ongoing consideration of the value of a more novel and cost-effective Genomic Resource Flock for Flystrike remains important.
The 10-year Merino Lifetime Productivity (MLP) project is capturing information on the lifetime performance of over 130 sires that are high and low for wrinkle, dags, breech cover, fleece weight, reproduction, fat and muscle, providing evidence of the productivity of naturally flystrike resistant Merinos. The project summary report, Breeding and Selection – Industry trends, also provides further information on this project.

ANALGESIA AND ANAESTHESIA

The development of practical analgesic and anaesthetic treatments for lambs has been a critical recent advance that alleviates pain associated with procedures such as mulesing and marking. Australian woolgrowers have been leaders in adopting anaesthesia and analgesia for husbandry procedures globally. More than 90% of respondents to a 2019 national online survey, undertaken by the University of New England (ON-00540), of Australian sheep producers to benchmark their 2018 parasite control practices, reported using pain relief (analgesics and/or anaesthetics) when mulesing their wether lambs, while 87% reported using pain relief with their ewe lambs. This is a significant increase from a similar 2014 survey, reporting on 2011 practices, (2014 Benchmarking Australian Sheep Parasite Control) in which 64% of respondents reported using pain relief when mulesing wether lambs and 59% in ewe lambs.

AWI has invested heavily in this area to assist with product development and make sure effective alternative anaesthesia and analgesia options are available for woolgrowers to use, and in education and extension to enhance uptake and use. A study, undertaken by Invetus and with input from RedCap Solutions (ON-00305 Metabolism study to determine the quantity and identify the nature of parent compounds and metabolites in ovine tissue and fluids following the topical administration of Tri-Solfen), provides supportive data to improve the characterisation of residues in edible tissues and as markers for analytical methods for each of the three active compounds in the anaesthetic Tri-Solfen (lignocaine, bupivacaine and cetrimide). Results from this work have been supplied to pharmaceutical companies and made publicly available for use in any applications to the APVMA using such compounds to reduce the withhold periods for these actives and to widen their use for their topical application in surgical wounds and injuries in sheep.

BREECH MODIFICATION PROCEDURES

Historically, AWI has also invested heavily in R&D into options for breech modification procedures to improve the lifetime resistance of sheep to flystrike. This has included the development of Clips, Skintraction and Liquid Nitrogen Process. Independent to AWI, AgVet Innovations has continued to further invest in the development and assessment of their Sheep Freeze Branding, that uses liquid nitrogen.

Additional activities in this area include research optimising mulesing for minimal welfare impact and information on performing the procedure: it includes the selection of lambs needing mulesing, the size of the procedure and optimal management during and following the procedure. What was the National Mulesing Accreditation Program (NMAP) manual has been updated by AWI with WoolProducers Australia and the Livestock Contractors Association and was recently published as the “Plan, Prepare and Conduct Best Welfare Practice Lamb Marking Procedures – Training Guide”. This training guide is designed to assist woolgrowers and their contractors perform lamb marking and mulesing procedures with the utmost care and attention to ensure the best short- and long-term welfare outcomes for the animal. The NMAP guide was only available to those completing NMAP training, however the new training guide is now freely available to all woolgrowers, as this seen as the best way forward to improve the on-farm husbandry practices for lamb marking and mulesing. This guide is also available to be used by any Registered Training Organisation to provide competency-based training for “Plan, Prepare and Conduct Mulesing Procedures” (AHCLSK334).

EDUCATION, EXTENSION AND PROMOTION

Communication with woolgrowers to inform them of the outcomes from flystrike R&D investment is a key focus of this pillar, ensuring they have the information they need to improve the lifetime welfare of their animals. Direct communication via the AWI newsletter, Beyond the Bale magazine, AWI website, project newsletters and woolgrower extension networks and workshops remain important, along with working with influencers of woolgrowers, such as their ram breeders, brokers, consultants, animal health companies and veterinarians (public/private sector). Along with the new "Plan, Prepare and Conduct Best Welfare Practice Lamb Marking Procedures – Training Guide" mentioned above, AWI continues to regularly distribute information for woolgrowers and their advisors on best practice flystrike prevention. Available on the AWI website at www.wool.com/flystrikelatest are recent flystrike prevention publications, including:

- Managing breech flystrike – 44-page manual (February 2019)
- Anaesthetics and analgesics including FAQs (December 2019)
- Tail docking – don't cut it short (March 2019)
- Premiums and discounts for mulesing status (September 2018).

ParaBoss, funded by AWI and MLA, and delivered by UNE, continues to regularly promote best practice advice on flystrike prevention through both FlyBoss (www.flyboss.com.au) and WormBoss (www.wormboss.com.au). In addition to its website(s), e-newsletter and Facebook page, ParaBoss in 2019 launched a series of podcasts (called Wormcasts) that included episodes relevant to flystrike prevention (www.paraboss.com.au/multimedia).

Figure 1. Information available for woolgrowers and their advisors on Dealing with Dag.

In 2012, an AWI-funded review on Minimising Dags in Sheep, provided useful information on the costs, reasons for and prevention of dag. Since the 2012 report was finalised, there has been further research undertaken in this complex area. The aim of a collaborative project (ON-00610), completed in 2019, was to review recent research outcomes on minimising dags in sheep and update the 2012 report, including the development of industry recommendations on the prevention of dags. Extension material produced from this project included a fact sheet (www.wool.com/dag-factsheet) and advisor manual (www.wool.com/dag-manual).

Measuring, monitoring and improving the success of education, training and extension programs in delivering on-farm husbandry practice change for breech flystrike is key to this pillar, enabling industry to better demonstrate its commitment to ensuring lifetime welfare of sheep. A 2017 AWI Merino Husbandry Practices Survey by Kynetec (available on www.wool.com/flystrikelatest) of Merino producers, to collect comprehensive benchmark data on their current practices, allowed for comparisons in animal husbandry practices between Merino types, states, regions, enterprise size and mulesed and non mulesed Merino enterprises. A 2019 Australian Sheep Parasite Survey (see ON-00540 University of New England), is the third in a series of surveys to measure change in parasite incidence and control practices, with previous similar surveys being undertaken in 2003 and 2011.

The industry is investing strongly in enhanced supply chain transparency and integrity. Communication and engagement with both domestic and international supply chain stakeholders has been steadily increasing each year. A critical component is the National Wool Declaration (NWD) program, ensuring transparency in the supply chain, by enabling informed wool purchasing decisions between the grower and buyer. The proportion of woolgrowers declaring their wool through the NWD continues to increase. The declaration by woolgrowers of their use of Analgesics and/or Anaesthetics (AA, previously Pain Relief) for mulesing is increasing, as is the proportion of Non Mulesed (NM) declarations (see Table 1).

Table 1. National Wool Declaration rates by Mulesing Status (Source: AWEX).

%	2008	2011	2017	2019 YTD* (2019/20)
Non Mulesed	3%	6%	12%	14.1%
Ceased Mulesed	3%	2%	3%	3.5%
Analgesic and/or Anaesthetic	3%	12%	32%	37.9%
Mulesed	29%	24%	20%	17.5%
Not Declared	62%	56%	34%	27.0%
Australian Clip	**38%**	**44%**	**66%**	**73.0%**

*Figures based on % sum of bales, all breeds and wool types, first-hand offered, P & D Certificates * YTD = as at 30 April 2020*

WHAT WE AIM TO ACHIEVE IN THE NEXT TWO YEARS

- Continue to monitor and define blowfly resistance to chemicals and refine blowfly chemical resistance best practice management advice.
- Continue to invest in early trials of new potential actives and control methods for parasite control treatments and vaccines, to the point where Pharmaceutical Companies have sufficient information to reasonably assess product development potential.
- Completion of a three-year long population study of blowflies to identify the extent of genetic differences in the fly across Australia to better manage the risk through effective integrated pest management and resistance management strategies and potential investigations into biological blowfly controls.
- Scope the development of a novel and cost-effective Genomic Resource Flock for Flystrike Resistance.
- Improved phenotyping and accuracy of animal welfare traits (breech, fat, worm resistance, lamb survival etc).
- Communicate the benefits, risks and timelines to breed for lower dags, breech wrinkle, breech cover and higher productivity, and continue to track these longer-term genetic trends.
- Further development, implementation, monitoring and evaluation of education, training and extension programs to ensure lifetime welfare of sheep, including on-going promotion of the use of analgesia and anaesthesia for surgical husbandry procedures.
- Engage woolgrower's advisors to ensure that they are kept abreast of the developments of the RD&E program.
- Further engagement with woolgrower groups, Government and welfare groups to ensure they are aware of the Flystrike Strategy, its outcomes and on farm changes and to provide opportunities for them to provide feedback on the Strategy and its direction.
- On-going independent assessments of the Flystrike Research, Development, Education, Extension and Communication Program.

FURTHER INFORMATION

Further information on the projects mentioned above, and many others, are addressed in the project summary reports or in project final reports available on the AWI website at www.wool.com/flystrikelatest. If you would like more information on any of the mentioned projects, email: bridget.peachey@wool.com or call 0429 006 527.

Bridget Peachey has been in her current position at AWI for 3.5 years, taking over from Geoff Lindon in late 2016. Prior to this, Bridget was the Policy Manager for Australian Lot Feeders' Association (ALFA).

Bridget graduated with a Master of Animal Science from Massey University in 1999 and has held a number of research roles in meat science and embryology, before working for Meat & Wool NZ (now Beef & Lamb NZ) as a Technical Trade Policy Analyst. She moved to Australia to take up a role with Dairy Australia as their Animal Husbandry Manager in 2006 before starting with ALFA in 2013.

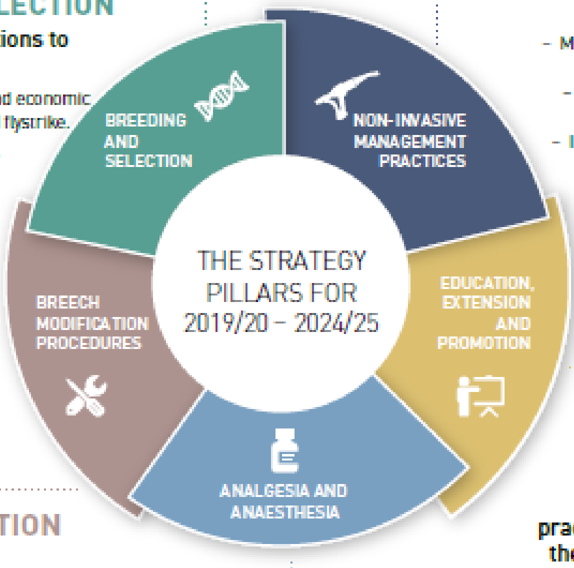
APPENDIX ONE

FLYSTRIKE RESEARCH, DEVELOPMENT, EDUCATION, EXTENSION AND COMMUNICATION STRATEGY 2019/20 TO 2024/25

Australian Wool Innovation Limited

VISION

ENSURE THE LIFETIME WELFARE AND PRODUCTIVITY OF SHEEP AND REDUCE THE RELIANCE ON MULESING.



BREEDING AND SELECTION

THE AIM: Long term solutions to advance lifetime welfare.

- Understand the performance and economic impacts of breeding for reduced flystrike.
- Investigate the, as yet unknown, factors that cause flystrike.
- Improve the accuracy of selection for flystrike resistance traits through phenotyping and genotyping.
- Better understand how to reduce the incidence of dags and urine stain through breeding.
- Track genetic trends for breech wrinkle, breech cover, dags and higher productivity.

NON-INVASIVE MANAGEMENT PRACTICES

THE AIM: Improved management practices to reduce the risk of flystrike.

- Monitor and define blowfly resistance to chemicals.
- Refine blowfly chemical resistance best practice management advice.
- Invest in early trials of new potential actives and parasitic control treatments and vaccines.
- Complete a population study of blowflies to identify potential genetic differences to inform blowfly management programs.

BREECH MODIFICATION PROCEDURES

THE AIM: Breech modification procedures to improve lifetime resistance to flystrike.

- Undertake further R&D of the animal welfare impacts of breech modification procedures.
- Undertake further R&D to refine the application protocols for breech modification procedures.
- Support best practice mulesing training.

EDUCATION, EXTENSION AND PROMOTION

THE AIM: Adoption of best practice strategies to improve the lifetime welfare of sheep, reduce reliance on mulesing and support transparency in the supply chain.

- Develop and implement education, training and extension strategies to improve lifetime welfare of sheep.
- Monitor, evaluate and improve the success of education, training and extension strategies.
- Engage with woolgrower advisors on the RD&E program.
- Ongoing engagement with domestic and international stakeholders to ensure they understand best practice management of flystrike and the welfare implications.

ANALGESIA AND ANAESTHESIA

THE AIM: Improved provision of analgesia and anaesthesia for surgical husbandry practices.

- Investigate longer acting, cost effective anaesthesia and analgesia options.
- Extend advice on analgesia and anaesthesia to woolgrowers.

Figure 2. AWI Flystrike Research, Development, Education, Extension and Communication Strategy 2019/20 to 2024/25.

A REVIEW OF PREDISPOSING RISK FACTORS FOR BREECH FLYSTRIKE

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PROJECT SUMMARY
Research over many years has focussed on the development of better methods of breech strike control. As a result, efficient fly control consists of an integrated approach which may include breeding for resistance, strategic crutching and shearing, prevention of scouring, strategic use of flytraps, paddock management to minimise strike risk and tail docking. However effective control of breech flystrike often relies primarily on mulesing and the prophylactic use of chemical insecticides with breeding for strike resistance a key element in an increasing number of flocks. Mulesing is exceptionally effective in preventing breech strike and has been the keystone procedure in integrated programs for flystrike control over many years but is increasingly untenable because of animal welfare concerns. In addition, resistance has emerged to the major chemical groups currently used for flystrike control, although the extent of this resistance amongst flocks is currently unclear. In this project the potential for new approaches to control was assessed.

This project, completed in 2019, was to undertake a comprehensive review of the predisposing risk factors for breech flystrike and report on recommendations for further flystrike research. The project:

- Reviewed Ausvet report documenting outcomes from a Flystrike Risk Factors Review Planning Meeting to summarise risk factors for flystrike and identify areas of knowledge deficit (see www.wool.com/flystrikecausalweb).
- Assisted in the planning, conduct and assessment of outcomes from a Breech Flystrike Review Workshop (4th December 2018, Stamford Plaza Sydney Airport) to identify areas of research and development priority.
- Reviewed AWI research conducted to date towards the identification of sheep risk factors for breech strike.
- Reviewed the literature on the role of odour in the development of breech strike and determining differences in strike susceptibility amongst sheep.
- Assessed a number of areas of interest flagged at the Flystrike Review Workshop, but not addressed in the main workshop recommendations.

RECOMMENDATIONS FROM REVIEW
Recommendations made from the different processes have been summarised below under three main headings: a) Breeding for resistance, (where a number of the areas were identified as high priority at the Breech Flystrike Review Workshop) b) Better understanding of the fundamental biology of *Lucilia cuprina* and strike development (also an outcome from the Flystrike Workshop); c) Other means of control. For a detailed description of the outcomes from this project, including a response to the recommendations from AWI, please see the project final report, available at www.wool.com/flystrikelatest. A number of these research areas are now currently being further addressed by AWI funded research programs or more detailed reviews to explore the opportunities in particular areas. Some of these are noted below.

Breeding for resistance

- *Increase the collection of phenotypic data from industry flocks (and other research flocks where relevant) with a view to the development of a breech strike index/indexes)*
  Encouragement of much more widespread phenotyping for flystrike traits is required to provide more robust and widely applicable estimates in Merino Genetics. This is particularly so for urine stain, which currently does not have a breeding value available in MERINOSELECT, and for scouring/dags. To this end there is a need to facilitate easier methods of measurement of 'difficult' traits such as urine stain and scouring/dags. This could be easier methods of assessing them, or perhaps indirect methods of estimating urine stain/risk of urine stain. The recording of alternative more readily measured estimates for the main flystrike traits e.g. faecal consistency for scouring, face cover for bare area, neck and body wrinkle for breech wrinkle for recording in MERINOSELECT and presentation of ASBVs for these traits should also be considered. Progeny testing of elite sires directly for breech strike incidence would provide an avenue for increased accuracy and maximising industry genetic gain in flystrike resistance.

- *Development of breech strike / welfare indexes.*
  There is a need to facilitate practical 'useability' of breech strike traits for sheep breeders in MERINOSELECT. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance that are specific to these different situations.

- *Better understand the unexplained variation in the occurrence of strike in resistant and susceptible sheep and the effect of management regime on this*
  The amount of variation in breech strike susceptibility not explained by the major indicator characters will be key to a consideration of the need for new or better indirect selection criteria. There is little unexplained variation in some data sets (e.g. crutched ewes in WA breech strike resource flocks where only 9.38% of the variation remains unexplained) and dags and skin wrinkles explain most of the phenotypic variation, as opposed to the NSW flocks and unmulesed, uncrutched flocks in WA where approximately 50% of the variation remains unexplained.

- *Invest in a genomics reference flock to generate genomics breeding values*
  This was considered a high priority, with a high cost, but high potential reward by Workshop participants. Genomic methods have major potential benefits for selecting flystrike resistance because sheep do not need to be exposed to strike or subject to the predisposing conditions for flystrike to enable selection and detailed and difficult phenotyping is not required. Rather, the genotype is estimated from a small blood sample. Furthermore, a genetic value can be attributed to all animals in all years and all environments, regardless of level of flystrike challenge. The establishment of a Genomics Implementation Working Group to determine the best path forward, with regard to available resources /resource constraints was recommended. This panel should include high-level specialist expertise in sheep/animal genomics, sound industry reference and representation from Sheep Genetics to facilitate implementation.

- *Support continuation of the breech strike resource flocks.* The two flocks provide a source of very accurately pedigreed and phenotyped animals and are in completely different environments with different flystrike profiles. The depth of phenotyping for flystrike incidence in the flystrike selection lines in WA (now at Katanning) and NSW (Chiswick) makes these flocks an important core resource for genomic studies, a prime resource for identifying and testing new indicator characters and valuable for obtaining more precise genetic parameters for the development of more accurate selection and breeding programs. The flocks would also be an important resource for research in other areas, for example investigating the role of microbiome profiles in strike etiology and susceptibility, testing the efficacy of new vaccine technologies and resistant phenotypes, and the future development of welfare indices (that incorporate resistance to breech strike) and breeding values.

- *Development of a detailed business case for investing in genetic improvement of sheep resistant to breech strike*
  To understand if further investment into breeding programs focussed on reducing breech flystrike is worthwhile, and to underpin promotion to woolgrowers about the application of genetic technologies or other approaches, an understanding of the size and scale of potential benefits is required – i.e. a value proposition/business case. A component of this work, for example, would be a benefit cost analysis for establishing genomic evaluation of flystrike'. This would also inform the feasibility/attractiveness of different approaches by quantifying the size of trade-offs that growers are willing to make.

Biology of *Lucilia cuprina* and strike development

- *Increase understanding of the fundamental biology of the Australian sheep blowfly*.
  In particular, to understand the genes that operate at different times in key developmental processes and in host detection, the location of suitable sites for oviposition and at different stages in the establishment of strikes by first instar larvae. This may assist in the development of improved bait or deterrent options and identify targets for new families of blowfly strike insecticides and vaccines. These studies need to be carefully targeted to provide knowledge with specific endpoints towards improving control efficacy and will be facilitated by the recent mapping of the sheep blowfly genome and advances in molecular technology. The need for a careful review of the abundant work already undertaken in this area before commencing new research was emphasised. The research needs to be targeted to specific outcomes in order to ensure efficiency and value of the investment. (Work in this area supported by AWI is underway, Project ON-00624 - Informed Development of a Blowfly Vaccine).

- *Understand the fleece / dag microbiome, and its role in breech strike susceptibility*
  It is well established that bacterial growth is important at various stages in the development of body strike, for example in providing odour cues for attraction and oviposition, causing skin scalding and extravasation which provides protein for the development of 1st instar larvae, and by providing a focus for skin invasion by newly hatched blowfly maggots. Microbial odours, particularly in association with urine or other decomposing organic matter have also been shown to be important in the attraction of other livestock ectoparasites to their hosts and bacteria often also provide critical nutritive factors for larval development of some livestock-associated flies.

  There has been much less study of the importance of the breech fleece microbiome and interactions with urine stain and scouring or of the importance of bacteria in the development of breech strike. However, there is indication that bacterial growth could be similarly important in determining breech strike susceptibility. The microbiome could also influence skin proteomic/metabolomic profiles and associated studies of the fleece/skin proteomics and metabolomics may yield additional important information towards the development of new approaches to control, for example vaccination against key bacteria, blocking bacterial odours, the use of bactericides or biological methods to control critical bacteria.

- *Better understand the role of attractants/odour in sheep susceptibility and the genesis of strike*
  Odour is involved at a number of stages during development of strike. In particular, the location of sheep, the identification of susceptible sites on sheep for oviposition and stimulating egg laying by flies. In sheep, odours associated with bacterial growth, particularly when in association with urine staining, scouring and diseases such as fleece rot and lumpy wool, are critical in determining susceptibility to strike. Many of the main odours involved at different stages appear to be bacterially and environmentally mediated and there is little evidence that innate (genetically controlled) odour differences between sheep influence fly attraction or are related to susceptibility. In addition, any innate differences in sheep odour are likely to be overwhelmed by the effects of bacterial odours during strike waves.

However, bacterial odours and other volatiles associated with predisposing causes of flystrike, such as urine and faecal staining, are critical to the initiation of strike and methods that interfere with the perception of odour by the flies, for example by targeting critical olfactory genes or processes, or the identification of strongly repellent molecules may lead to novel control approaches. Studies in this area should take account that odour could be operating at a number of stages in strike development in addition to attraction (for example acting as an arrestant or egg laying stimulant) and design experimental tests accordingly. Clarification of olfactory mechanisms in *L. cuprina* and the genetic basis underlying these may lead to the identification of new insecticide or vaccine targets, novel compounds with persistent repellent modes of action or which work by disrupting egg laying by the flies.

Other means of control

- *Manage insecticide resistance and maintain the efficacy of available flystrike control products*
  The availability of effective flystrike protection and treatment chemicals remains critical to effective management of flystrike in Australian flocks, particularly in non-mulesed flocks. There is a long history of resistance development to flystrike control chemicals in *L. cuprina* populations and the recent emergence of resistance to the keystone control products, dicyclanil and cyromazine is a major threat to sustainability of wool production. (See AWI project ON-00491 Sheep Blowfly Resistance Update). This will be particularly important in unmulesed flocks, highly susceptible flocks and flocks in high flystrike risk regions. It is currently unclear whether or not resistance is widespread and how many flocks are affected.

  There has been limited detailed consideration of the optimal design for long term resistance management programs. A project to investigate different resistance management approaches is required. The possibility of developing products based on chemical mixtures, a strategy widely used for combating resistance to gastrointestinal parasites but not currently used for flystrike control should be considered.

- *Develop new insecticidal actives for flystrike control*
  With increasing costs of development and registration, the rate of new sheep blowfly insecticides coming onto the market has “slowed to a trickle”. The sheep parasiticide market is relatively small in the world context and this is particularly relevant as most of the major pharmaceutical companies that conduct research in this area have a multinational focus. Research in this area, desirably in partnership with commercial AgPharma companies, will assist the continued availability of effective flystrike preventatives for use by Australian woolgrowers. The availability of the *L. cuprina* genome provides the possibility of new insecticidal targets and AWI is currently funding a project in this area (ON-00454 New Chemicals for Flystrike Control). There may also be an opportunity to consider other previously tested compounds known to be effective against *L. cuprina* but not developed for this use to date because of prevailing market circumstances.

- *Development of flystrike vaccines*
  AWI projects towards the development of a flystrike vaccine are currently underway (AWI Project ON-00619 Vaccine for Control of Flystrike). This is a high risk, but potentially very high reward area which will be facilitated by the recent availability of the *L. cuprina* genome. A vaccine directed against fleece rot bacteria, critical in susceptibility to bodystrike was previously developed and patented, but never commercialised (Burrell 1985). This vaccine gave extended protection against fleece rot and bodystrike. As preliminary evidence suggests that many of the same bacteria may be important in susceptibility to breech strike, investigation of the potential of this vaccine for use in reducing susceptibility to breech strike may be worthwhile. AWI Project ON-00723 is currently reviewing the feasibility of fleece rot and lumpy wool vaccines.

- *Reducing the incidence of scouring*
  Scouring (diarrhoea) and resultant dags in the breech wool of sheep are major predisposing causes for breech strike in the southern sheep production areas of Australia. Dags are also a major management issue

in their own right in these areas. Methods to reduce the incidence of scouring and dags would have a major impact in reducing breech strike incidence. Recommendations towards the reduction of dags have been provided to AWI in a previous project (AWI Project WP520 and subsequently updated (AWI Project ON-00610 Minimising Dags in Sheep). A fact sheet (www.wool.com/dag-factsheet) and advisor manual (www.wool.com/dag-manual) on managing dag have recently been published.

- *Biological control of sheep blowflies*
  Biological control could be either classical biological control, which consists of the release of specialist natural enemies that are expected to persist in blowfly populations keeping fly populations low (classical biological control), or innundative biological control (biopesticides) where large numbers of pathogenic organisms (fungi, bacteria, viruses), parasites or predators are released, or used to treat sheep, as 'biological pesticides'. *L. cuprina* occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites is almost invariably density-dependent. This factor and the lag time generally experienced between a pest outbreak and a corresponding increase in numbers of biocontrol agents would seem to present difficulties for classical biocontrol agents to persist and impact on *L. cuprina* populations, or more particularly, to reduce strike incidence.

  Biopesticides such as *Bacillus thuringiensis* and some entomopathogenic fungi have shown short term protection when applied to sheep in experimental studies and suitable agents may have application as part of an integrated approach or in organic flocks. However, they are unlikely to provide a level or persistence of protection comparable with chemical pesticides, which limits their practicality in many situations. Pathogens that persist in the soil, such as some fungi or entomopathogenic nematodes, may have effect against soil stages of *L. cuprina* (prepupal larvae and pupae) particularly during the overwintering phase, better knowledge of the spatial and temporal ecology of the soil phases of *L. cuprina* will be required to assess whether sufficient mortality could be induced to significantly affect flystrike incidence. One particular type of soil fungus, *Tolypocladium cylindrosporum* was shown to cause high mortality in the soil stages of *L. cuprina* in NZ and the potential for biological control of *Lucilia spp*. using sheep blowfly pathogens was reviewed in more detail as part of AWI Project ON-00620 Review of Sheep Blowfly Pathogen Control. *Tolypocladium cylindrosporum's* prevalence in Australia is currently being investigated (ON-00721 Identifying cylindrosporum in Australia).

- *Area wide genetic controls for Lucilia cuprina*
  These methods seek to bring about suppression or eradication of the target pest by the release of insects of the same species that have been modified to confer sterility or cause genetic death in the pest population. This approach is also known as autocidal control and could be used in area wide strategies focussed on eradicating sheep blowflies from an area or suppressing fly abundance through ongoing releases of modified flies. The most well-known method, the sterile insect technique (SIT) was successfully used to eradicate screwworm flies (a cattle pest with similar biology to sheep blowflies) from North and Central America, as well as to eradicate an exotic incursion of screwworm flies in Libya and which is also used for eradicating regional incursions of fruit flies in fruit fly-free areas of Australia.

  The availability of gene editing technologies such as CAS CRISPR provide the potential for more elegant systems of genetic control such as RIDL (Release of Insects with Dominant Lethality) or potentially using gene drives to spread deleterious genes (often sex-linked or stage specific genes) through fly populations. The previously noted research currently being funded by AWI, to identify critical genes in *L. cuprina* may facilitate the design of genetically modified strains suitable for use in area wide autocidal approaches. Transgenic sexing "male only" strains have been developed in North American *L. cuprina* strains and consideration should be given to the feasibility of the future use of these strains in the design of area wide strategies in Australia.

FURTHER INFORMATION

For a detailed description of the outcomes from this project, including a response to the recommendations from AWI, please see the project final report, available at www.wool.com/flystrikelatest.

REFERENCE

Burrell, D. H. 1985. Immunization of sheep against experimental pseudomonas-aeruginosa dermatitis and fleece-rot associated body strike. *Australian Veterinary Journal* 62: 55-57.

Dr Peter James' main area of interest is in new technologies and integrated approaches to the control of pests and parasites associated with domestic livestock species. He has worked with ectoparasites and nuisance insects associated with most of Australia's major livestock industries and involvement with research and extension on sheep ectoparasites extends over more than 30 years. Formally at SARDI and the Roseworthy Campus of University of Adelaide in SA, he is currently a Senior Research Fellow at the Queensland Alliance for Agriculture and Food Innovation (QAAFI) at the University of Queensland. Peter's PhD was on the biology and population dynamics of sheep lice, he was leader of the team that developed LiceBoss, and he is a member of the ParaBoss Technical Advisory Committee.

SHEEP BLOWFLY RESISTANCE UPDATE

AUTHOR
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SUMMARY
The impact of insecticide resistance on flystrike is usually a reduction in the protection period provided by treatments rather than complete control failure. The blowfly that initiates most flystrike in Australia is *Lucilia cuprina* which has developed widespread, high-level, stable resistances to insecticides like the organophosphates. To provide up to date information on the presence of resistance, the level of resistance and its distribution across the sheep producing areas of Australia, this project investigated six insecticides registered for flystrike control. The six insecticides included 1) diazinon[a], which is representative of the Organophosphate group; 2) ivermectin[b] a macrocyclic lactone (ML); 3) spinosad[c] a spinosyns; 4) imidacloprid[d] a noenicitinoids; 5) cyromazine[e] a triazine and 6) dicyclanil[f] a pyrimidine derivative, the final two belonging to the IGR group.

This study provides an update on previous studies which did not detect resistance to spinosad (2002) or ivermectin (2002) but did find cases of low-level resistance to dicyclanil and cyromazine (2012-14) [(1)]. Also, initial data were collected on imidacloprid, which has only recently been released for flystrike prevention. Spinosad, ivermectin and imidacloprid are also used to control the sheep biting louse and incidental exposure of the blowfly could increase selection pressure and provide additional opportunity for the development of resistance.

While individual submitters received the resistance profile for their property, this project identified the need for, and informed on the development of, an integrated resistance management plan for flystrike control across Australia.

Example products: [a] Coopers Diazinon™ [b] Coopers Blowfly and Lice™; [c] Extinosad™; [d] Avenge + Fly™; [e] Vetrazin™; [f] CLiK™

METHODOLOGY
Approximately 455 maggot collection kits were distributed, upon request, across Australia. To date 122 submissions have been received of which 101 were *Lucilia cuprina* maggots that survived transport and were reared for testing. To date, the resistance profiles of 87 viable submissions have been reported back to their submitters. These were from NSW (n=42), Western Australia (n=21), South Australia (n=12), Victoria (n=11) and Tasmania (n=1). There have not been any submissions from Queensland (see Figure 1).

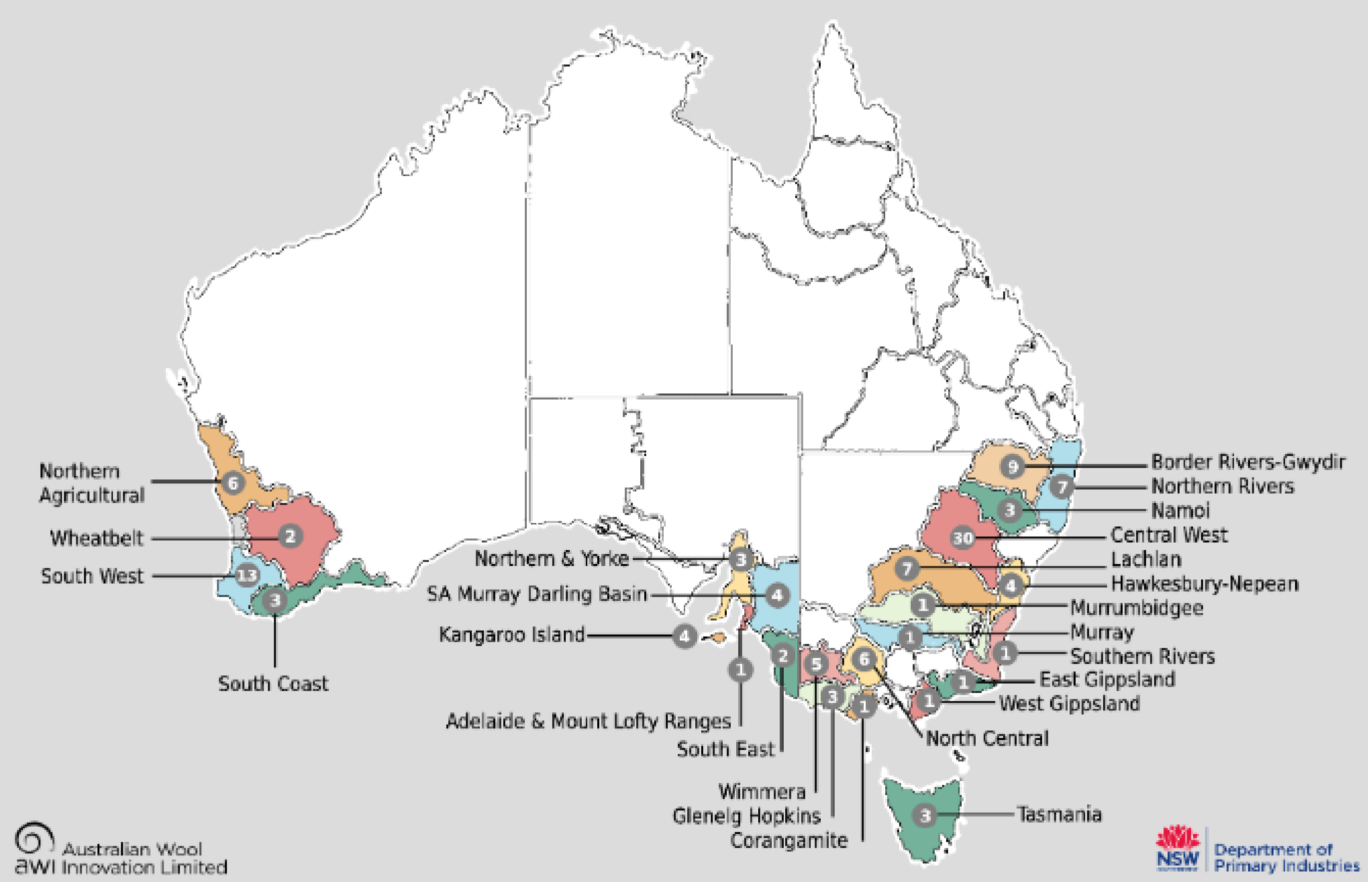

Figure 1. Number of maggot submissions by region.

FINDINGS

To enable comparison between submitted blowfly strains for each insecticide, a resistance ratio (RR) was calculated for each strain relative to a susceptible reference strain. Here we report the project findings to date on 87 completed submissions. Analysis of the final data set will be required to determine the statistical significance.

1. Regardless of the state of origin, and despite decreased use of OPs for many years, all submissions were resistant to the OP diazinon as expected. A strain from Tasmania had the minimum RR of 8-fold while the maximum RR of 51-fold was a strain from NSW. Comparison of these RR ranges with those determined in 1985 (5 to 60-fold)[(2)] provide further evidence of the stabilisation of OP resistance.
2. A significant increase in the range of response of blowfly strains to ivermectin has occurred since 2002[(3)] when RR's ranged from 0.6- to 2.8-fold (n=74) while this study determined RR's ranging from 1.3-fold to 9.2-fold.
3. The response of current NSW submissions to spinosad (n=42) with those reported in 2002[(4)] show the range of RR's has also increased from 0.2- to 2.6-fold in 2002 (n=31) to 0.4- to 4.6-fold in 2020.
4. For many years imidacloprid has been used for lice control but only since 2019 for flystrike. Base line data is usually collected prior to extensive use of an insecticide. In the absence of base line data on imidacloprid, this study reports the current range of response which cannot be assumed to be a susceptible range. The range of RRs is large, being from 3.2-fold (Tas) to 42.5-fold (Vic). Further categorization is required but this data will act as a benchmark for the future.
5. Low level resistance to cyromazine (without dicyclanil resistance) was found in 48% of strains from WA (n=21), 33% from South Australia (n=12) and 9% from Victoria (n=11). The percentage of cyromazine only resistant maggots ranged from "present but below 1%" to 73%.
6. To date 100% of submissions from NSW have concurrent dicyclanil and cyromazine resistance (n=42). This is not the case for other states with only 29% from WA (n=21), 25% from South Australia (n=12) and 82% from Victoria (n=11). In individual strains the percentage of dicyclanil resistant maggots ranged from 2% to 93%.

The high prevalence of resistance to dicyclanil and cyromazine in NSW submissions (100%) prompted a trial which determined the % reduction in protection provided by currently marketed products against strike by highly dicyclanil resistant maggots. A 50mg/L dicyclanil spray-on product had protection reduced by 78% while the 12.5g/l and 65g/l dicyclanil based spray-on products had 73% and 69% reductions respectively. Jetting fluids with cyromazine and ivermectin as actives had protection periods reduced by 50% and 33% respectively. Against maggots which were susceptible to dicyclanil all of the products protected for the periods listed on the product labels.

The ability of dressing products to kill full grown, dicyclanil resistant and dicyclanil susceptible maggots was determined in vitro. The ranking of products from most effective to least effective are as follows:

a) Dicyclanil susceptible: Flystrike Powder > Spinosad aerosol > ivermectin jetting fluid > cyromazine jetting fluid > Diazinon > Propetamphos > Spinosad jetting fluid.
b) Dicyclanil resistant: Spinosad aerosol > Ivermectin jetting fluid > Cyromazine jetting fluid > Flystrike powder > Spinosad jetting fluid > Propetamphos > Diazinon.

KEY MESSAGES

The widespread drought conditions determined the number and location of origin of the maggot submissions we received. The most submissions were received from NSW which all displayed resistance to both cyromazine and dicyclanil. We determined that dicyclanil resistance greatly reduced protection, allowing maggots to form strikes many weeks earlier than claimed by the five products tested. Producers should note that the labels of dicyclanil based products have been modified to claim protection from flystrike "caused by dicyclanil-susceptible strains of blowflies.". These products performed according to the label claims against dicyclanil susceptible maggots. Under favourable flystrike conditions dicyclanil resistance may govern management practices across the production year. Alternatives to a single treatment with a dicyclanil based product may be required to protect sheep across the spring through to autumn fly seasons. If dicyclanil resistance is on your property, rotate to other insecticide groups. In WA, SA, and Victoria, where there appears to be lower levels and lower frequencies of dicyclanil resistance, producers still have the opportunity to decrease selection pressure. Producers should adopt an integrated resistance management plan based on rotation between insecticide groups to reduce the selection of resistance and protect the effective life of flystrike products.

Spinosad, imidacloprid and ivermectin are also registered to prevent flystrike and belong to different insecticide groups. Products based on these actives provide shorter periods of protection than dicyclanil but can still be used effectively, for example, late in the fly season or prior to crutching or shearing. Use a dressing product from a different insecticide group to the one used to prevent flystrike and also rotate groups if you treat twice in the year. Weigh sheep and follow the label instructions as under-dosing and overdosing provide the opportunity to select for, or increase the levels of, resistance.

There are non-insecticidal flystrike tools like breeding sheep that are less prone to flystrike. Until this process is successful some form of breech modification may be required. By knowing the highest flystrike risk periods on your property, you can strategically time drenching, crutching and shearing so that minimum wool length and/or clean breeches occur at those times. A flystrike calendar can be developed for your property using the FlyBoss Tools which uses data from your closest weather station. An integrated flystrike management plan will provide protection for your flocks when needed, allow other activities such as cropping to be undertaken and ultimately be cost effective.

FURTHER INFORMATION

1) "Resistance Management Strategy for the Australian Sheep Blowfly (*Lucilia cuprina*)" (April 2019) FlyBoss. http://www.flyboss.com.au/sheep-goats/files/pages/treatment/insecticide-resistance/resistance-management-strategies/190415-SHEEP-BLOWFLY-RESISTANCE-MANAGEMENT-STRATEGY-FINAL-GD3349.pdf

2) "A Fly in the ointment" (December 2019) FlyBoss. http://www.flyboss.com.au/sheep-goats/files/pages/treatment/insecticide-resistance/resistance-management-strategies/A-Fly-in-the-Ointment-Managing-Chemical-Resistance-to-Blowflies-20191129.pdf
3) Sales, Suann and Koeford (2020) "Dicyclanil Resistance in the Australian Sheep Blowfly, *Lucilia cuprina*, Substantially Reduces Flystrike Protection by Dicyclanil and Cyromazine Based Products." International Journal for Parasitology: Drugs and Drug Resistance. (Accepted: in press).

ACKNOWLEDGEMENTS
Monica Suann, Blake Brangwin and Kim Koeford.

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(1) Levot G., Resistance to flystrike preventative treatments. AWI Breech Strike R&D Technical Update 2014.
(2) Hughes and Levot (1985) Final Report WRTF Project K/1/1103.
(3) Levot and Sales (2002) Australian Journal of Entomology 41, 75-78.
(4) Levot and Sales (2002) Australian Journal of Entomology 41, 79-81.

Narelle Sales - Professional Officer (Research) NSW DPI - Elizabeth Macarthur Agricultural Institute.

Narelle has conducted research on endo- and ecto-parasites of livestock for many years. Since joining NSW DPI, she has worked on industry funded research projects mainly on the Australian Sheep Blowfly, Lucilia cuprina*, and the sheep biting louse,* Bovicola ovis*. This has included large scale trials, pen trials and predominantly laboratory studies on insecticide resistance. Narelle also develops and then utilises techniques for research and diagnostic use in the areas of immunology, serology, molecular biology, bacteriology, as well as parasitology to service Australian agricultural industries.*

ADDENDUM TO AWI PROJECT NO: ON-00491 SHEEP BLOWFLY RESISTANCE UPDATE

AUTHOR
Dr Jane Littlejohn, AWI General Manager Research

COMMENT
AWI has funded a series of blowfly resistance research with NSWDPI which is described in past published papers. Points about the NSWDPI methodology covering a larval implant technique, and the production and use of pure-breeding blowfly strains, as quoted directly from these published papers, is presented in this addendum. It is hoped these quotes provide some context to assist the reader in drawing conclusions from the current work. The preceding work, including the "Nimmitabel strain" and the "Nimmitabel Selected (NS) strain" was published as:

- Levot GW. Response to laboratory selection with cyromazine and susceptibility to alternative insecticides in sheep blowfly larvae from the New South Wales Monaro. *Aust Vet J* 2013; 91:61-64.
- Levot GW, Langfield BJ, Aiken DJ. Survival advantage of cyromazine-resistant sheep blowfly larvae on dicyclanil- and cyromazine-treated Merinos. *Aust Vet J* 2014; 92:421-426.

<u>The larval implant technique</u>
This research technique for indicating resistance is when larvae, from the pure-breeding blowfly strains being studied, were implanted on the skin of research sheep, in 7 months wool, which were treated with chemical products.

"The larval implant technique aims to control all variables other than the effect of the insecticide treatment, but is not perfect."

"Whereas hand-jetting places insecticide onto the skin where blowfly larvae feed, spray-on products that are deposited onto the tip of the wool staple rely on formulation characteristics and rainfall to move the chemical to the skin level. Until this translocation of insecticide occurs, the larval implant technique, which places larvae at skin level, could produce erroneous results because the larvae would not contact the insecticide."

"Similarly, there is a greater likelihood of sub-optimal application with low-dose spray-on products than with the high volumes of jetting solutions."

"These factors may explain the anomalous strikes recorded on single sheep in the spray-on cyromazine and dicyclanil groups at 5 and 8 weeks, respectively. The larval implant technique aims to control all variables other than the effect of the insecticide treatment but is not perfect. Probably because of inadequate wetting of the fleeces, the F2011 controls failed at week 11 and although unfortunate, this single-point anomaly does not detract from the overall trend of the NS strain forming strikes sooner after treatment than the F2011 strain."

"With regards to the larval implant model, Hughes and Shanahan considered an implant to be positive if even a single feeding larva was present at the 24 hour post treatment inspection and a treatment to have failed when

two sheep of the five sheep in a treatment group sustained strikes. This seems a very conservative approach, but by these criteria, the spray-on and liquid cyromazine formulations failed to protect sheep from strike by the NS (Cyromazine resistant) strain by 8 weeks after treatment. Protection for <8weeks is considerably shorter than the 11 (Vetrazin Spray-on) and 14 weeks (Vetrazin Liquid) claimed on the label of these and similar generic products and demonstrates how sustained selection pressure on low-level resistant field populations could curtail the protection offered by cyromazine based products".

<u>Pure-breeding blowfly strains</u>
The larvae from the pure-breeding blowfly strains for the larval implant technique were bred by the researcher from the suspected resistant maggots which made up 4% of maggots originally collected by the farmer. The researcher reared each of generation of the maggots on known doses of chemical and bred them through 13 lifecycles to ensure they are "pure-bred" for resistance.

"By rearing the original Nimmitabel larvae on homogenized liver containing the susceptible discriminating concentration of cyromazine, a pure-breeding strain comprising only the 4% resistant individuals from the original population was created in the laboratory."

"The original cyromazine-resistant 'Nimmitabel' strain was reared for 13 generations on homogenized liver containing cyromazine at a concentration lethal to susceptible larvae."

"The 'Nimmitabel' strain responded to laboratory selection by becoming more resistant to cyromazine (8x) and to dicyclanil (3x)."

"The blowflies used for the implants were a composite strain called 'F2011' comprising the descendents of field-collected blowflies that were susceptible to cyromazine and the pure-breeding, cyromazine-resistant NS strain."

CONCLUSIONS
Inferences from findings in a research setting were the foundation of general messages to sheep producers on the potential for resistance emergence and development and its management.

"Resistance, even in the pure-breeding resistant strain, was not so severe as to cause treatment failure with cyromazine or dicyclanil, but was sufficient to reduce the protection period provided. It is recommended that producers adopt management practices that minimize the development of resistance to these and other compounds."

"As far as we know, populations as resistant as the NS strain do not currently exist in the field. However, such populations must be considered a possible consequence of prolonged or frequent exposure of low-level resistant larvae to concentrations of cyromazine or dicyclanil that differentially favour survival of resistant phenotypes over susceptible types."

"Whether the survival advantage possessed by the NS strain poses a real risk on-farm, however, depends on whether resistant blowflies and flystrike-susceptible sheep are likely to coexist during the critical intervals when resistant larvae can survive on treated sheep but susceptible types cannot."

"Considering the demonstrated reduction in flystrike protection provided by these insecticides against the NS strain, it would be prudent to reduce further selection for resistance by adopting practices that preserve the current levels of susceptibility to these and other compounds. Where possible, these should include fewer insecticide applications, no second treatments with the same or a related compound in the same wool growing cycle, strategic use of non-chemical management strategies such as shearing and crutching, and a longer term move towards plainer bodied sheep that are less susceptible to flystrike and require fewer insecticide treatments."

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GENETICS OF BLOWFLY PARASITISM & CRISPR – PHASE 2

AUTHOR
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SUMMARY
Understanding the biology of the Australian sheep blowfly is critical to developing new and effective strategies for its control. The unique parasitic lifecycle of the blowfly means that insect model organisms currently available for study using sophisticated genetic techniques are not necessarily going to provide results about gene function relevant to the blowfly.

The research project, **ON-00373 Genetics of Blowfly Parasitism,** aimed to improve on the genomics resources available in *Lucilia cuprina* (2015-2018) because, while the initial genome was of a high quality when released, new sequencing technologies could be used to improve the *L. cuprina* genome further. This would assist researchers to correctly identify genes, and gene families and provide a blueprint for researchers to analyse the function of genes. To perform such functional analyses, researchers required a way to manipulate genes in the blowfly. A parallel project, **ON-00516 CRISPR, (2018-2019)** used some of the improved genomic resources to develop a method that would efficiently allow researchers to edit the blowfly genome and facilitate the analysis of genes that could help in the search for new blowfly control options.

PROJECT REPORT
In order to understand the biology underlying blowfly parasitism and to assist researchers in their efforts to identify weaknesses in the blowfly that could be targeted to exert control, an improved genomic blueprint and enhanced genetic tools were required. There has been a huge improvement in the technologies available to sequence and assemble genomes and we used one of these to create a higher quality *L. cuprina* genome assembly for researchers to work with. Prior to this project the methods available to investigate the biology of the blowfly and to understand the genes and proteins critical for their capacity to parasitize sheep lacked power. Clustered random interspaced short palindromic repeat (CRISPR) genome editing technology had proved to be a highly efficient and effective strategy for engineering a variety of mutations into genes and was being utilised in several insect pests. Given that no options were available for generating mutations in genes of interest in blowflies, developing CRISPR for use in the blowfly was seen as a priority.

OUTCOME/REPORT
The major aim for the **ON-00373 Genetics of Blowfly Parasitism** was to assemble and annotate an improved genome. Genome sequencing included use of a new sequencing and assembly technology, Genomics Chicago® library preparation with HiRise™ genome assembly, with one of the initial developers of the method, Dovetail™ Genomics. The table included below outlines the features of the new genome compared to the initial genome published in 2015. The average length of DNA scaffolds is far higher for Freeze 2. This means that the full genome sequence is represented in far fewer large scaffolds, rather than many small fragments. This improved continuity of the genome sequence assists with annotation of complete genes as well as providing information in regards to gene order. In this context, note the N50 and N90 values which show the number of scaffolds that

include 50% and 90% of the complete genome assembly, respectively. BUSCO = Benchmarking Universal Single-Copy Orthologues and is a set of genes that is used as a measure of genome quality/completeness and we again see that in the new Freeze 2 genome there are more complete genes and less fragmented or missing genes.

Table 1. Features of the new genome compared to the initial genome published in 2015.

Features of the Genome assemblies	2015 genome (458 Mb)	Freeze 2 genome (465 Mb)
N50 length (bp); Scaffolds required to reach 50% coverage	744,413bp; **165 Scaffolds**	6,922,854bp; **18 Scaffolds**
N90 length (bp); Scaffolds required to reach 90% coverage	126,471bp; **736 Scaffolds**	1,321,550bp; **83 Scaffolds**
BUSCO complete genes; BUSCO fragmented genes; BUSCO missing genes	2594; 52; **153**	2704; 47; **48**

N50 = 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value

Following assembly of the Freeze 2 genome it was annotated to identify genes on the scaffolds. The number of predicted genes had decreased to 12,933 genes (Table 2). The number of genes without orthologues (counterparts in other species), i.e. genes considered to be unique to the blowfly, has also decreased substantially. This is mainly due to the large number of new insect genomes sequenced since the first release of the blowfly genome in 2015.

Table 2. Gene prediction comparison between the 2015

Gene Prediction Comparison	2015 Genome (14,544 genes)	2018 Genome (12,933 genes)
Single-copy orthologues (4 spp.)	4,106 genes	4,425 genes
Single-copy orthologues (1 sp.)	12,160 genes	11,142 genes
Genes unique to the blowfly	2,062 genes	572 genes

Gene expression was analysed by sequencing RNA from different tissues of the blowfly including odour sensing organs (antennae and maxillary palp). Sequencing was also performed on larvae that had hatched and developed on sheep, prior to initiating a wound. This gene profiling provided support for the gene annotations in the updated genome. We began to use the genomic and transcriptomic resources created in this project to investigate blowfly biology during the detection and invasion stages of blowfly parasitism of sheep. The odorant receptor (OR) genes of *L. cuprina* were extracted from the newly assembled genome. The two tissue specific transcriptomes, one for the antennae and maxillary palp and the other for blowfly wings were sequenced and expression differences in OR expression examined. We found that there are no odorant receptor genes that have expression exclusively in either males vs females or in females that are mated or not. We expanded our analysis to include chemosensory receptors and some of these did show differences associated with the diet that adult flies were reared on. Follow up work on these genes could include further sampling to test a greater range of conditions helping to validate these findings and work on identifying the odorants and the nature of the responses blowflies have when they are detected (i.e. behaviours such as attraction).

Some of these data generated in this project have led to a new collaboration on olfaction being formed between Prof Coral Warr at UTAS and Prof Batterham and Dr Perry at UOM. This research project examining the evolution of olfactory receptors in *L. cuprina* will run from 2020-2022 and has been funded under the Australian Research Council Discovery Project scheme. It will continue the work looking at odours and odorant receptors that were involved in the attraction of blowflies to sheep. This would not have been possible without the genomic data provided by the ON-00373 project and the development of the CRISPR/CAS9 methodology and is an example of how results from projects funded by AWI have helped attract new researchers, built the capacity of researchers to investigate blowfly biology and led to new projects bringing early career researchers and students to the field.

Another focus of our bioinformatic analyses was to identify genes that were expressed early in the development of larvae on sheep. Assays using gene knockdown highlighted the potential for some genes to halt larval development. These preliminary data were encouraging and can be followed up with the more precisely gene editing technology that we have now developed. Some of this analysis is being used to help identify useful candidates for development of blowfly control options such as a vaccine in the current **ON-00624 Informed Development of a Flystrike Vaccine** project.

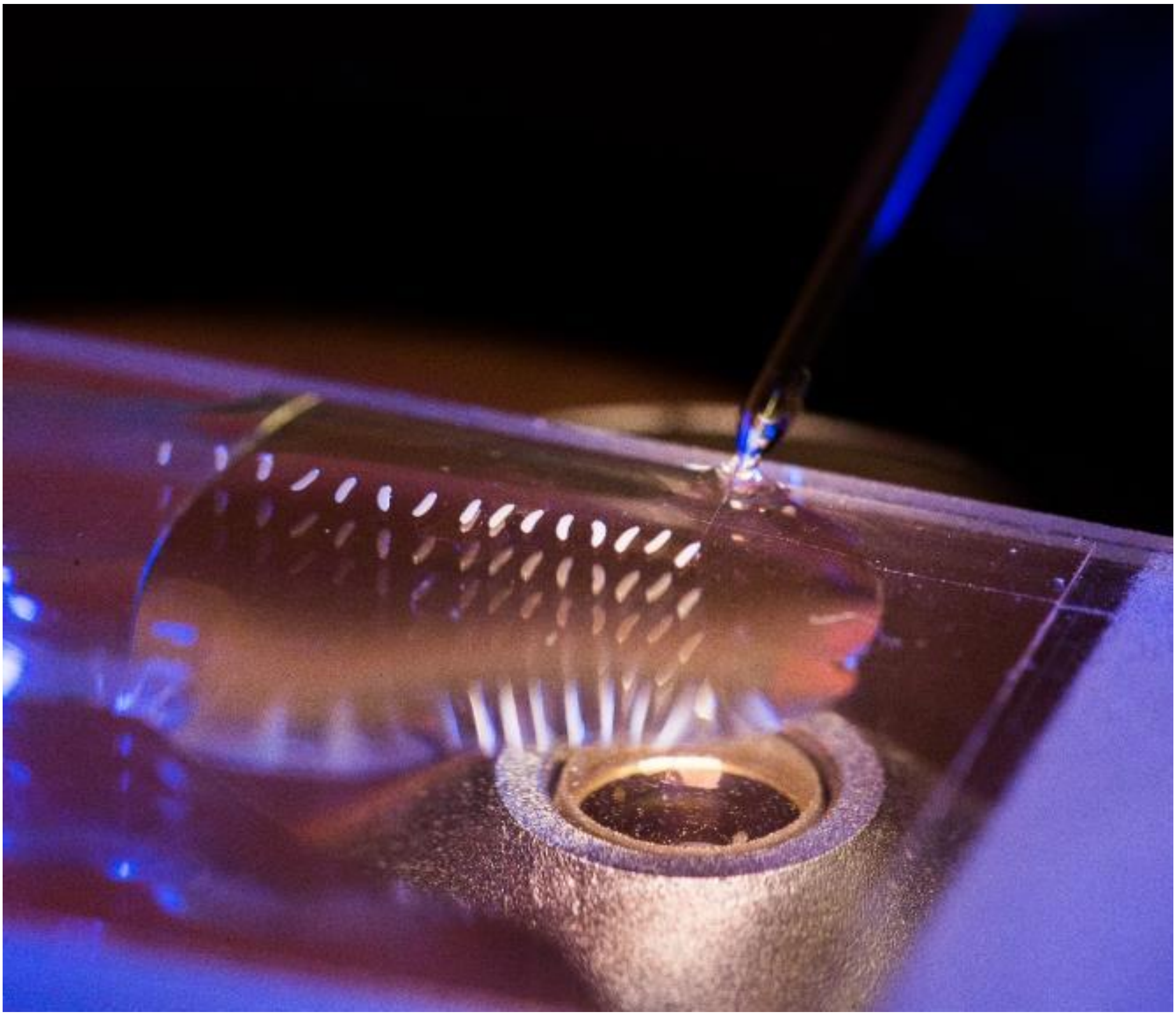

Figure 1. Microinjection of fly embryos to introduce the CRISPR/CAS9 components required to modify their genome.

Our results from the CRISPR/CAS9 project (**ON-00516 CRISPR Phase 2**) were promising. We tested multiple methods of microinjection to introduce different combinations of DNA, RNA and Protein into fly embryos which led to the successful creation of targeted deletions. Germline events were identified in two genes, *white* and *Orco* that we then confirmed by sequencing. We were able to establish multiple fly colonies carrying deletions in the *white* gene. The selection of the *white* gene as a target in this project was a strategic decision as this provides an easily identifiable visible marker (flies have white instead of red eyes – pictured below) that we aim to use in future work, including further development of the technology. While the flies carrying the mutation in the *Orco* gene were less fit, we have also been able to establish a colony of flies homozygous for the *Orco* deletion. These flies are unable to produce a functional *Orco* protein and lack the capacity to smell. These will continue to be studied through the newly funded ARC Discovery project, the foundation of which was is based on taking this research forward.

Figure 2. CRISPR/CAS 9 gene modification technology was used to knock out the eye colour gene of the sheep blowfly, producing a fly with white eyes.

PROJECT RECOMMENDATIONS

As reported previously, we have presented the current approach and also future ideas on identifying vaccine candidates to two companies. In both instances it was clear that the project was at too early a stage to attract a commercial partner. There is a need for further biological evidence of a particular gene target being useful as a vaccine candidate to make a compelling case for commercial investment in this area. The vaccine candidate list assembled and analysed in this project was thus at too early a point in the validation process for commercialisation. It does have the potential to be commercially valuable with some further analysis and biological validation that would refine the list to a small number of key candidate genes and this is being pursued as part of the current **ON-00624 Informed Development of a Flystrike Vaccine** project.

The use of CRISPR will only increase in the insect field and we would strongly recommend maintaining a close eye on developments in this space. An additional benefit is that we have also found that being able to perform functional analysis in *L. cuprina* has increased student interest in pursuing blowfly research projects with our groups which will enhance the ability to recruit new scientists to the field.

These two projects were led by a team of academics across the School of BioSciences, (Dr Trent Perry and Professor Philip Batterham), and the School of Veterinary Sciences, (Prof Robin Gasser, Dr Clare Anstead and A/Prof Vern Bowles) at the University of Melbourne. Dr Perry and Prof Batterham are molecular geneticists with experience working on insecticide resistance mechanisms and functional genetics, including the use of genome editing technologies. Dr Anstead led the analysis of the Lucilia cuprina *genome and, together with Prof Gasser, specialises in genome assembly and annotation. A/Prof Bowles has extensive experience in parasitology, insecticides and vaccine research.*

INFORMED DEVELOPMENT OF A BLOWFLY VACCINE

AUTHOR

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SUMMARY

This research project (2019-2022) focuses on the genetic analysis of Australian sheep blowfly populations across the country. Results will provide critical information to support efforts in identifying and assessing potential blowfly larval proteins for their development as flystrike vaccine candidates. We are assisting CSIRO researchers (ON-00619) to analyse their potential vaccine candidates while also examining our latest results for novel larval vaccine candidates. In addition to vaccine development, results from this study will provide data for cost-benefit assessments of various flystrike control strategies and could also assist with planning their implementation. The genomic analysis of flies from around Australia will be scanned for the presence of genetic changes that could be associated with insecticide resistance against several of the current chemical classes used to treat flystrike.

PROJECT REPORT

Current blowfly control relies heavily on surgical and chemical treatments. A desire to develop novel options that could replace mulesing and address the risk of insecticide resistance requires a detailed understanding of the fly's biology and an in-depth analysis of its population genetics.
Over the four years of this project we are focussing on two main areas:

1. Using genetic and genomic data to determine the structure of blowfly populations in Australia and assess the level of genetic variation that exists within and between the locations from where they are collected.
2. Understanding the molecular processes that allow the maggots to survive on sheep.

The genomic data will contribute to several different areas related to blowfly control. Knowledge gained from both the population and molecular analyses will be used to identify proteins which have the potential to be developed as a flystrike vaccine. Some of our research is already being used, in collaboration with CSIRO, to help refine the selection and prioritisation of vaccine candidate proteins for vaccine trials (see report on project ON-00619 Vaccine for Control of Flystrike).

The genomic data will also provide critical information about fly movement around the country which will subsequently help to predict the spread of insecticide resistance outbreaks and assist in informing the development of regional resistance management plans. We are also mining the sequencing results for the presence of insecticide resistance alleles in fly samples. This could provide an early warning of where insecticide resistance may be an issue in the future.

Figure 1. Dr Anstead out and about delivering and setting up blowfly traps for the 2019/20 season in Western Australia.

Our first aim to *determine the structure of blowfly populations in Australia and assess the level of existing genetic variation* has progressed well; trapping was conducted over 62 sites in the initial collection season. Flies were collected from properties in QLD, SA, TAS, NSW and VIC and constituted the first of three rounds of collections. The results from the analysis of the initial dataset were very encouraging and genetic markers were identified that will be used to distinguish fly populations from different regions. There were clear distinctions between QLD, TAS and other regions and preliminary analysis suggests that we are also detecting the migration of flies between some regions of VIC, SA and NSW. Future sampling will provide the resolution needed to develop our blowfly population models. There are not enough samples at this stage to make precise conclusions; however, with the population sampling just completed in the 2019/20 and more to be made in the 2020/21 season, we will be able to provide a clear direction on whether populations are distinct, and which populations are isolated from each other, which has implications for management of resistance outbreaks.

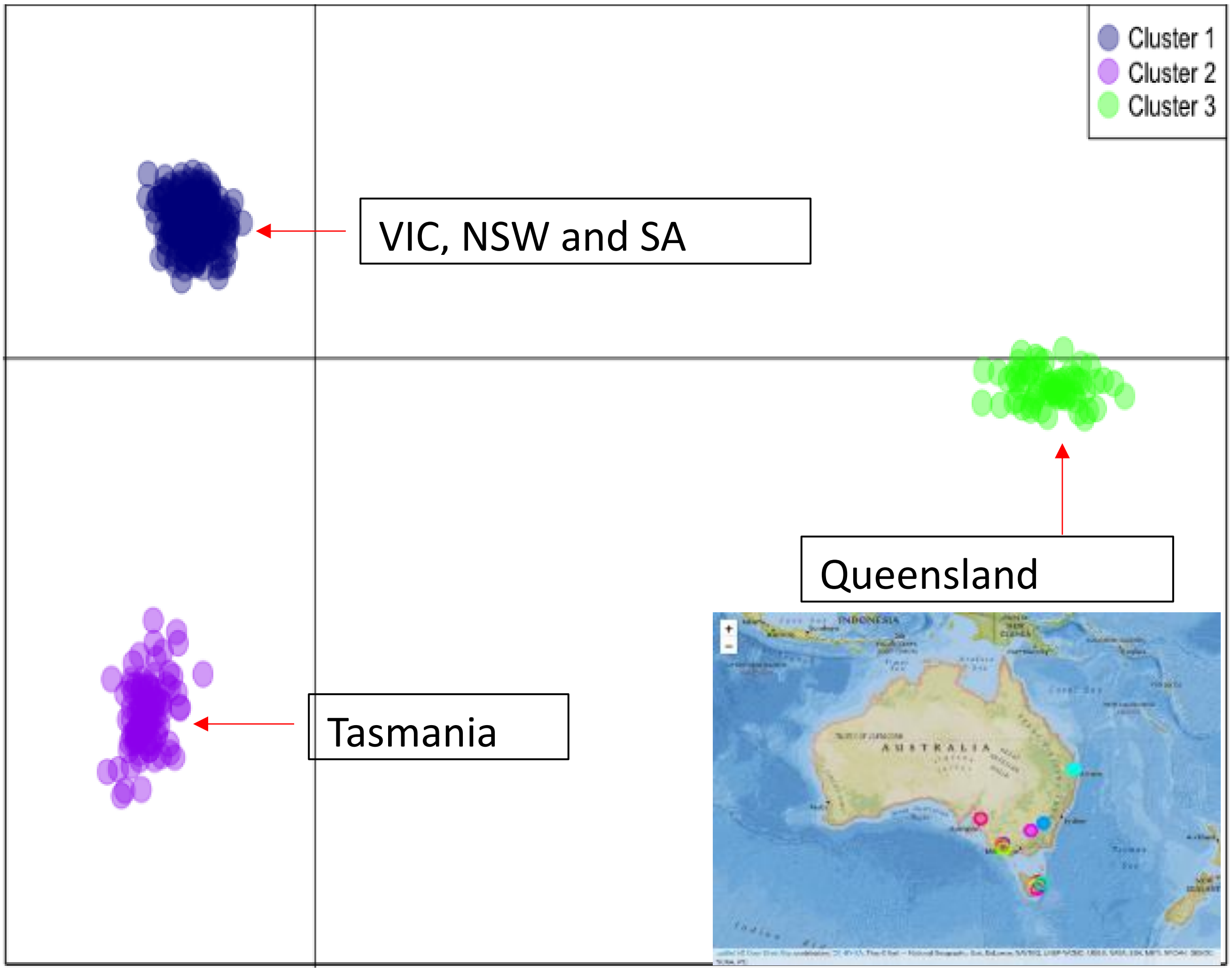

Figure 2. Discriminant Analysis of Principal Components (DAPC) plot with the blowfly collection sites. Populations of blowflies are clearly divided into three distinct clusters and this type of analysis will help us look at dispersal, gene flow and admixture between the populations in each cluster.

As part of our collaboration with CSIRO, our genetic data is being applied to help prioritise their vaccine candidate antigens and is also being used in UoM's efforts to identify new potential vaccine candidate genes. We use the genomic data from pooled blowfly samples to check for genetic differences present in different sample areas in order to identify which larval proteins would be best selected for a vaccine that would be effective against all sheep blowfly populations across Australia.

Following ethics approval in the first half of 2019, significant progress towards our second objective*, understanding the molecular processes that allow the maggots to survive on sheep,* has been made. An *in vivo* sheep implant study was conducted in June and July 2019. Blood serum, larval tissue, larval excretions and sheep wound exudate samples were collected. Wound biopsy samples were also collected for subsequent analysis. Proteomic analysis of larval excretion samples has since been conducted on several samples to identify the proteins excreted by maggots during the early stages of wound initiation. We have identified several hundred maggot peptides present in these samples and have been matching these to our data on gene expression that was obtained during the ON-00373 project. Further analysis later this year will continue to build a detailed picture of the early phase of flystrike which will assist in refining our candidate selection and help understand the biological pathways that are involved.

Regular face-to-face meetings with the research team at CSIRO led by Dr Tony Vuocolo (pre-COVID19) have been held since the initiation of the two projects. Both groups have been working together on evaluating and refining the candidate selection and our data has been used to provide input into the results from trials currently being run by CSIRO. We will be continuing to work together in 2020 to further refine the list of proteins that have the potential to be developed as vaccine candidates.

We welcomed Dr Shilpa Kapoor to the research team in September (2019). Dr Kapoor brings highly relevant experience in parasitology, next-generation sequencing and population genetics to the project team. Since joining the UoM team, Shilpa has made an excellent contribution in analysing the 2019 population genomic data, as well as beginning to establish some of the bioinformatics analysis workflows that will be used later in the project. Field sampling of blowflies from the 2019/20 season have also been completed. Hundreds of flies from across the country have been identified and are about to undergo variant analysis. The new data will be integrated with our initial samples to help us build our blowfly population models over the next 12 months.

WHERE TO NEXT/NEXT STEPS

In the period ahead we will be focussing on our research and look forward to continuing to collaborate with CSIRO to support their vaccine trials. We have been fortunate with timing in that our collection of flies was close to complete prior to the disruption of COVID19 and we have been able to continue our analysis. Our final blowfly collection is planned for September this year and we will again be looking forward to working with growers to assist in obtaining samples from across the country, particularly in areas we have not previously trapped flies. We have greatly appreciated the support and efforts of all of the growers in providing samples for this study.

The research at University of Melbourne is being led by a team of academics across the School of BioSciences, Dr Trent Perry, and the School of Veterinary Sciences, Dr Clare Anstead and A/Prof Vern Bowles. Dr Perry is a molecular geneticist with a background in insecticide resistance and functional genetics. Dr Anstead, a parasitologist specialising in genomics and bioinformatics, led the initial Lucilia cuprina *genome project released in 2015. A/Prof Bowles has vast experience in parasitology, insecticides and sheep parasite vaccine research. Dr Shilpa Kapoor recently joined the team, bringing further parasitology and bioinformatic expertise.*

VACCINE FOR CONTROL OF FLYSTRIKE

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SUMMARY
CSIRO with support from AWI has just completed its first 12 months of research in developing a vaccine to control flystrike. During this initial period, specific classes of antigens have been identified and 26 individual antigens produced as recombinant proteins in bacterial or insect cell expression systems. These prototype vaccines are currently undergoing testing in vaccine trials in sheep and being assessed for immunological response and efficacy in controlling flystrike. There are more antigens in the pipeline that are being developed and novel approaches using synthetic biology being investigated. The next 12 months will provide important information derived from these vaccine trials and identify lead priorities for the next step in this technically challenging initiative.

CSIRO acknowledges its productive collaborations with the University of Melbourne, Griffith University and the University of Queensland Protein Expression Facility in this research endeavour.

PROJECT BACKGROUND
CSIRO is proud to partner with AWI in a project aiming to develop a Flystrike Vaccine for the Australian sheep and wool industries. Flystrike, and its control, costs the industry dearly each year (>$170m p.a.) and the industry is facing mounting hurdles in the effective and socially acceptable process of combating this pest. The industry needs an effective, welfare-friendly alternative flystrike control and prevention technology. CSIRO has a long history in ectoparasite vaccine research, developing a commercial cattle tick vaccine and undertaking foundation research in buffalo fly, screwworm fly and sheep blowfly vaccine research in the 1990's to early 2000's. The strategic investment by AWI and research undertaken by the University of Melbourne in sequencing the sheep blowfly genome has created new opportunities in better understanding the biology of this pest. With the advent of new technologies and the genome information, significant new opportunities now exist that is allowing CSIRO to build on its foundation flystrike vaccine knowledge and helping make the development of a flystrike vaccine a real possibility.

Development of a Flystrike Vaccine is a high-risk venture with complex technical hurdles to overcome, however it offers the real potential of a new paradigm for flystrike control for the producer that will garner the support of consumers, retailers and animal welfare advocate organisations, thereby contributing to the future success, profitability and sustainability of the sheep wool and meat industry. In addition, a flystrike vaccine will fulfil a wide range of requirements by providing whole animal protection against body and breech-strike, reducing the use and reliance on chemical insecticides and overcome the need for the practice of breech modification. The other advantage a vaccine will provide is that it can also be used as part of an integrated pest management approach that will allow reduced insecticide use and help prolong the utility of this control measure.

Since the start of the project, 14 months ago, CSIRO has been working in collaboration with the University of Melbourne (Perry and Anstead Group), Griffith University (Kolarich Glycomics Group) and the University of Queensland Protein Expression Facility utilising their respective expertise to assist in the development of prototype vaccines for testing in sheep for flystrike control. Vaccine trials in sheep are currently underway to test these prototype vaccines with more research and testing still to be done. The following is a brief description of what has and will happen with the research project over its initial 3-year period.

GOALS AND OBJECTIVES OF THE PROJECT

Key project objectives are to:

- Identify a pipeline for antigen identification and production.
- Produce prioritised vaccine antigens as recombinant proteins in bacteria, yeast or insect cell systems or by chemical synthesis.
- Test recombinant protein antigens in prototype vaccines in sheep trials.
- Identify vaccine antigens that induce strong immune responses in sheep and produce inhibition of *Lucilia cuprina* larval growth *in vitro*.
- Identify lead vaccine antigens to progress to testing and commercial development of a flystrike vaccine with an Animal Health Veterinary Pharmaceutical partner.

WHAT HAS BEEN ACHIEVED BY THE PROJECT TO DATE?

Candidate vaccine antigen identification

Sheep blowfly larvae are incredibly tough and resilient organisms and there is limited opportunity to target the larvae through an immunological approach using a vaccine. The larvae are coated in an incredibly tough waxy cuticle whilst the foregut and hindgut are lined by a tough impermeable polysaccharide polymer called chitin. The most susceptible region of the larvae is the midgut that is devoid of this chitin lining but lined instead by a secreted semi-permeable membranous matrix called the peritrophic matrix, a stocking-like structure. CSIRO established that certain classes of protein that help make up this membrane, when used as antigens in a vaccine, produce an immune response in sheep that significantly inhibits larval growth. The discerned mode of action of this vaccine approach is directing antibodies to key proteins contained within the peritrophic matrix and blocking it. This blocking mechanism restricts the secretion of proteases into the midgut lumen, inhibits the passage of nutrients to the underlying midgut microvilli, thereby starving the larvae and inhibiting their growth and viability. Additionally, targeting excretory and secretory proteases, enzymes that are crucial to larval food digestion and establishment on the skin of the sheep may have potential as vaccine antigens and help promote the growth inhibition described.

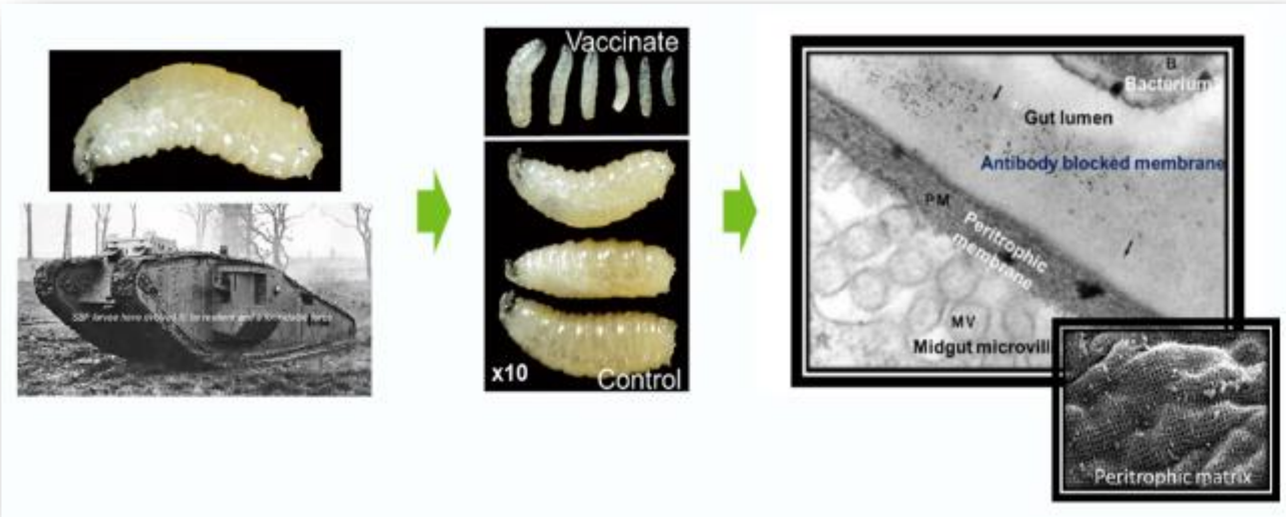

Figure 1. Sheep blowfly larvae are tough and resilient organisms and finding a susceptible region in their armour is key to vaccine development. Key midgut associated proteins have been used and demonstrated to be good targets for vaccine development. Vaccination with these proteins as antigens has resulted in larvae growth inhibition by immunological blocking and interference of the peritrophic matrix lining the midgut.

Using Next Generation Sequencing technology and the blowfly genome sequence, a sequencing approach has been used to identify all the genes that are active in the blowfly larval lifestages and in the key larval tissues, the cardia and the salivary gland. This process has enabled identification of the complete repertoire of the cardia expressed peritrophic matrix associated proteins. The salivary gland gene expression analysis has also provided information on excretory and secretory proteins that are produced by larvae. Together with blowfly lifestage gene expression analysis, candidate protein-encoding genes have been identified that are now being investigated as vaccine antigens.

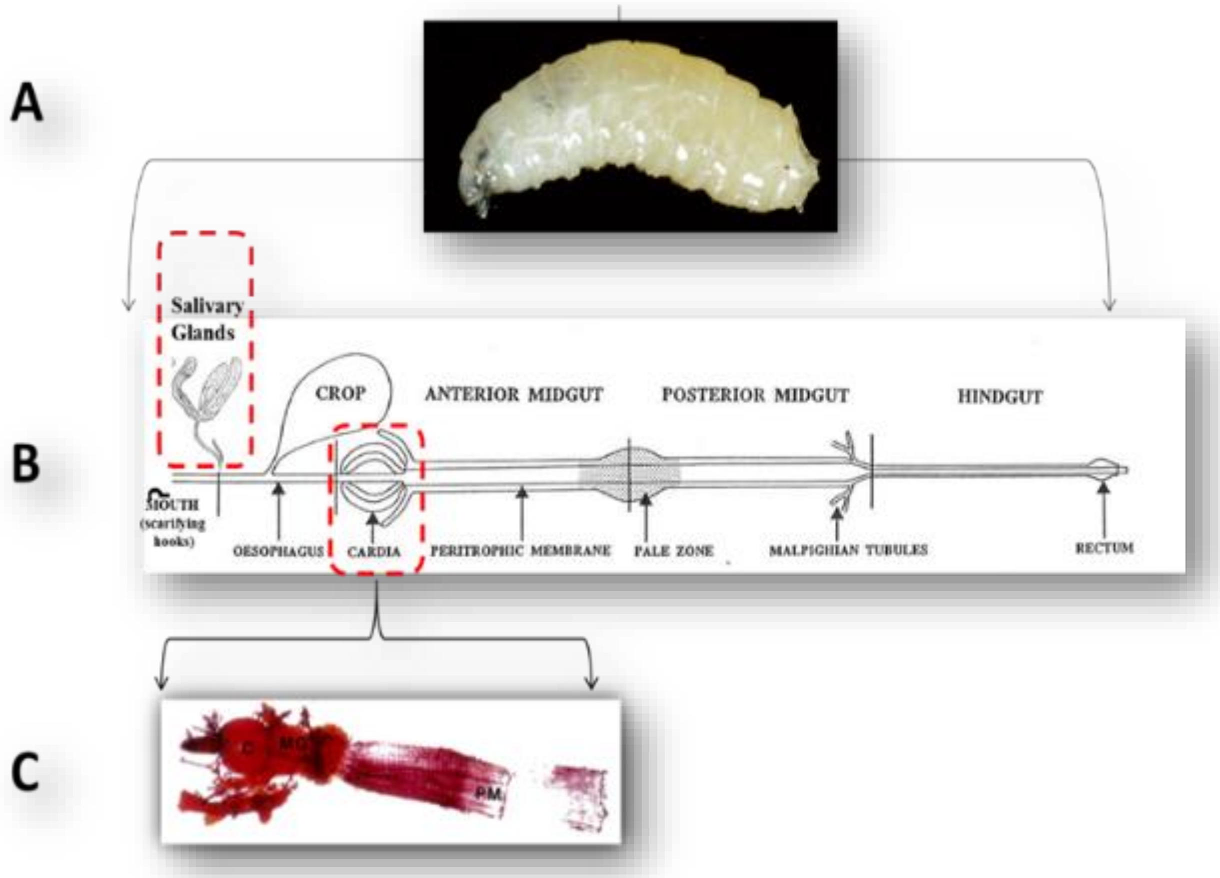

Figure 2. (A and B) Blowfly lifestages and key tissues (salivary gland, cardia and midgut) have been sequenced to identifying activated genes encoding proteins that are secreted and potentially accessible to immunological targeting by sheep antibodies when larvae start to feed on sheep. (C) Cardia (C) and anterior midgut (MG) cultured in the lab showing the peritrophic matrix (PM) that they produce. The PM represents a key target for immunological targeting a potential source of vaccine antigens.

Candidate vaccine antigen production

Lead candidate antigens representing key protein classes identified from the tissue and lifestage gene expression analyses are being tested as prototype vaccines. These antigens have been engineered and produced as proteins using recombinant molecular technologies utilising either Bacteria or Insect Cells as "protein production factories". Twenty-six recombinant antigens have been engineered, cloned, expressed, purified and formulated with adjuvants to produce prototype vaccines and are at different stages of testing and validation as prototype vaccines tested in sheep. During and at conclusion of the trial, blood is collected and sera assayed for immunological response and ability to confer protection from flystrike initiation and larval growth.

Figure 3. Key steps in the process of engineering and formulation of candidate antigens for prototype vaccine production and testing. Steps involve engineering the candidate gene encoding the protein target of the vaccine, cloning it into bacteria or insect cells, culturing the cells to produce the recombinant protein/antigen, purifying the antigen, formulating it with an adjuvant and administering it to sheep which are then assessed for antibody titre response to the vaccine and larval growth effects.

Sheep immunological studies have also been undertaken to investigate immunological response of sheep to control antigens simulating a flystrike vaccine. This is informing the effect of vaccine dose and longevity of the immunological response and assisting with formulation of the trial flystrike vaccines. In addition, studies in collaboration with Griffith University Glycomic's Institute are underway to investigate the chemical synthesis and modification of vaccine antigens to modulate and improve the immune response of sheep to the vaccines.

Figure 4. CSIRO research flock at the Chiswick research laboratory and farm are used for vaccine testing.

Figure 5. CSIRO research team undertaking tasks associated with flystrike vaccine development.

WHAT DO WE ENVISAGE THE PROJECT WILL BE UNDERTAKING IN THE NEAR TO MID FUTURE?

Stage two of the vaccine project involves further refining and testing of lead vaccine antigens with the aim to deliver a validated short-list of candidate antigens that when formulated into prototype vaccines prevent or inhibit flystrike establishment and larval growth. Information garnered from this project will help inform future research into flystrike vaccine development. CSIRO will continue to work together with collaborators at University of Melbourne and further refine candidate antigen selection.

Engineering and producing highly effective Flystrike Vaccine antigens will require refining the protein production system or developing new protein production tools to achieve this critical need. This rationale is core to the collaboration CSIRO is forging with Griffith University researchers in developing synthetic biology approaches to vaccine development and exploring novel cell lines for production of recombinant antigens.

CSIRO aims to deliver by the completion of Stage Two, refined and validated lead vaccine antigens that when formulated into prototype vaccines prevent or inhibit flystrike establishment and larval growth. We have strong interest in this project from VetPharma companies and once a validated short-list of candidate antigens is identified, discussions with a commercial partner will be progressed with the key aim of commercial development of a flystrike vaccine.

Dr Tony Vuocolo is a Senior Scientist and Flystrike Vaccine Project Leader at CSIRO Agriculture and Food, leading the AWI and CSIRO funded research project developing a vaccine to protect sheep from flystrike. He has a 30 year history in livestock production and health research with many publications in these areas. Tony is a molecular biologist with extensive expertise in research and development of vaccines against a variety of ectoparasites. His research experience and key skills are in molecular biology, protein chemistry, genomics, epigenomics and immunology. Email: tony.vuocolo@csiro.au

NANOTECHNOLOGY FOR FLYSTRIKE AND LICE CONTROL

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SUMMARY
New chemical formulations for flystrike control are required to support the phaseout of mulesing and because of the development of resistance to the most widely used flystrike control compounds. Control of sheep lice has suffered similar resistance problems and remains an issue in the sheep industries. Nanotechnology offers a means of providing extended and 'softer' protection of sheep against flystrike and lice. This project is designing and testing unique silica nanocapsule formulations with spikes on the particle surface and purpose-designed release characteristics to give prolonged periods of protection against flystrike and lice, with minimal residues and off-target effects. This will provide new, labour efficient, options for managing flystrike in unmulesed sheep and countering resistance in sheep blowflies and lice.

Background
With ongoing requirements to increase production efficiency and constraints on the availability of labour livestock producers increasingly favour parasite treatments that can provide extended periods of protection. For this reason there has been much interest in controlled release technology such as long-acting injectable formulations for internal and blood feeding ectoparasite control, slow release polymer matrix devices such as ear tags and collars for prolonged buffalo fly control in cattle and flea control in dogs and cats, rumen capsules for helminth and tick control and more recently, microencapsulated and nanoparticle formulations.

Whereas traditional formulations of pesticide depend for prolonged action on a single initial high level treatment so that control is maintained until concentrations decay below effective levels, controlled release systems aim to release pesticides in steady amounts at active levels or to release only at times of infestation risk. This approach has a number of advantages in addition to prolonged control. Doses need not be as large so there is less risk of tissue residues. There is generally a lower risk to the operator and of environmental contamination and there is a reduced chance of subclinical toxicity or accidental poisoning of animals. In addition, there are a number of 'softer' chemistries, including plant extracts that have been shown to have activity against *Lucilia* spp. These compounds are often favoured in pest control, particularly by organic producers, because of their rapid degradation in the environment and lower potential for tissue residues but are of limited practical usefulness because of their limited persistence. Suitable controlled release systems may enable the use of insecticides which have not previously been suitable for use because of poor persistence in the fleece. Micro or nanoencapsulation technology can protect these compounds against environmental degradation and release them strategically at times of flystrike risk, or over an extended period of time to provide practically significant periods of protection against flystrike.

A wider choice of insecticides would be valuable in providing additional options in planning insecticide usage programs to minimise resistance development. In addition, controlled release systems that maintain insecticide at high concentration and then give a rapid residue decay avoiding resistance-selecting 'decay tails' (Anderson et al 1989), particulate controlled release system that could sit inert in the fleece and only release in the presence of moisture, systems that maintain high levels of insecticide through the fly season and then decay during the winter when no flies are present, or systems containing insecticides that degrade rapidly once released could also reduce the risk of resistance development.

Major innovations in the area of nanotechnology have led to the development of a variety of nanoparticle-based pesticide formulations, including polymeric/cellulose nanocrystals and lipid nanoparticles. By encapsulating active ingredients into nanocapsules, breakdown due to environmental pesticides can be reduced and chemical can be delivered at steady active levels over a prolonged period or designed to release only at times and sites where they are needed. Nanoencapsulated formulations also have the important attribute that they can generally be applied using existing application equipment.

UQ Silica nanoparticles

The UQ silica nanoparticles are a patented technology to fabricate novel hollow silica (SiO_2) nanocapsules that can be loaded with active molecules to enable superior protection against insect pests (Australian Patent Appl No. 2015901379). The nanocapsules have a large hollow cavity and porous silica shell which protects the internal active payload against degradation, while pores in the shell allow easy active loading into the hollow cavity and sustained release of the active compound. A number of designs of particle have been tested. The basic design is the smooth nanoparticle (SNP) as described above. However, a number of more recent designs of rough-surface nanoparticles (RNP) have a more pollen grain like topology (Figure 1a) with silica spikes (or 'whiskers') covering the nanocapsule outer surface. Similar to pollen grains, these spikes aid retention of the capsules on surfaces. The characteristics of these particles are 'tunable' and the particles can be designed with different characteristics such as with different chemical payloads, different size, different wall thicknesses and pore sizes, and different silica 'whisker' characteristics to optimise their functionality for different uses. This project is developing and testing silica nanocapsule formulations that can potentially provide prolonged, safe and residue free protection against sheep flystrike and lice and provide new, labour efficient, options for managing these pests. The UQ nanocapsules also possess advantages compared to other types of nanoparticles for translation to a viable commercial product. Polymer or lipid nanocapsules are often expensive or unstable under field conditions, whereas silica has been well recognized as inert and abundant in the environment with good bio-compatibility and is approved by Food and Drug Administration (FDA) for oral delivery. Moreover, the UQ patented technology provides a relatively simple approach to the fabrication of nanocapsules, employing cheap industrial chemicals, which is ideal for large scale commercial oriented production.

Three types of silica nanoparticles were initially studied in this project, smooth surface silica nanoparticles (SNP's), silica nanoparticles with silica spikes on the surface (RNP's) and RNPs with a surface modification to provide hydrophobic surface characteristic (RNP-C18) (Figure 1). The initial particles were 200-300 nm in diameter, but a number of other diameter particles with diameter from 180 – 800 nm have been fabricated and tested.

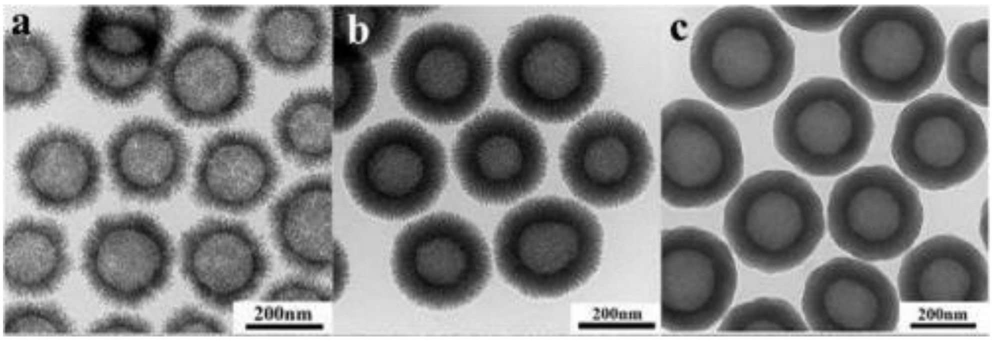

Figure 1. Transmission electron micrograph images of (a) rough nanoparticles; (b) rough particles after C18 surface modification and (c) smooth nanoparticles.

More recently, two new types of particles (FNS-60 and FNS-60-H) with-hydrophobic surface characteristics have been developed and are being tested. The FSN-60 particles have a higher pore volume than the previous formulations allowing higher chemical loading which, depending on release dynamics, is expected to provide further improvements in longevity of effect.

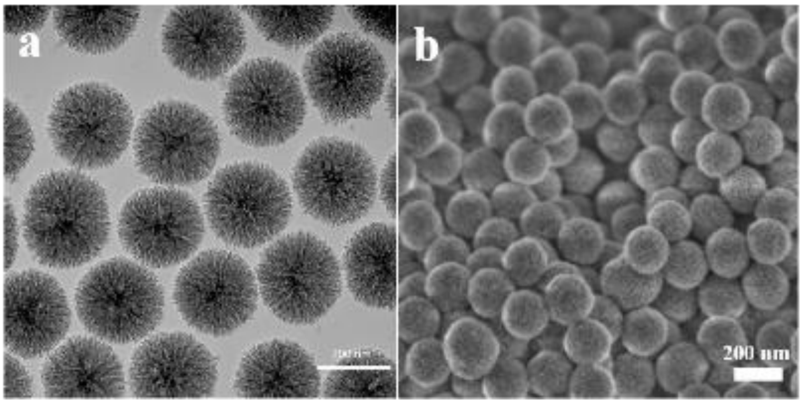

Figure 2. a) Transmission electron micrograph and, b) scanning electron micrograph images of the FSN-60 silica nanoparticles.

As noted above, it is expected that the silica nanoparticles will be able to provide greater persistence of protection by protecting encapsulated chemicals from environmental breakdown and in the case of the rough nanoparticle types, superior adherence to wool and to the cuticle of insects. Adherence to wool fibres is shown below. The electron micrographs (Figure 3) show the nanoparticles adhering to the wool fibres after water rinsing. This effect appears to be most marked with the C18 nanoparticles (Figure 3c) with the remaining particles more evident than with the smooth and rough particles.

Figure 3. Electron microscope images of nanoparticles adhered to wool after water rinsing; (a) smooth nanoparticles (b) rough nanoparticles (c)RNP- C 18 nanoparticles.

We have also investigated the distribution and cuticular adherence of the different silica nanoparticles following treatment of *L. cuprina* larvae using fluorescence microscopy. Blowfly larvae were exposed to the fluorescein-labelled nanoparticles using a standard larval wool assay whereas sheep body lice were exposed by either being placed in wool that had been dipped in the nanoparticle solutions or by exposing them to a lice diet that had been treated with the nanoparticles. Figure 4 shows a high density of fluorescein-labelled particles (RNP) in the guts of both first stage blowfly maggots and lice. This indicates that both the insects are ingesting significant amounts of the labelled particles. The feeding habits of both insects would seem to favour active accumulation of particles but whether the particles are attaching to gut lining or peritrophic membrane, or just accumulating as the insects feed is currently unclear.

Cuticular adhesion was also noted in the assays with both blowfly larvae and lice, but the fluorescence was much lower, than in the gut. This is expected as ingestion of particles occurs actively as the insects feed whereas the particles on the cuticle would be acquired passively and presumably more slowly as the larvae or lice contact particles as they move through the wool or on the skin surface. Cuticular electron micrographs for both blowflies and lice suggest that the C18 and rough nanoparticles both adhere more strongly to cuticle than the smooth particles and that the C18 particles adhere more strongly than the rough particles. These results suggest that best effect against both blowfly larvae and lice is likely to be achieved when the nanoparticles are administered with the objective of oral toxicity. However, the rough or C18 particles could also be expected to add to the toxic dose delivered, particularly with purpose designed chemical payload and release characteristics.

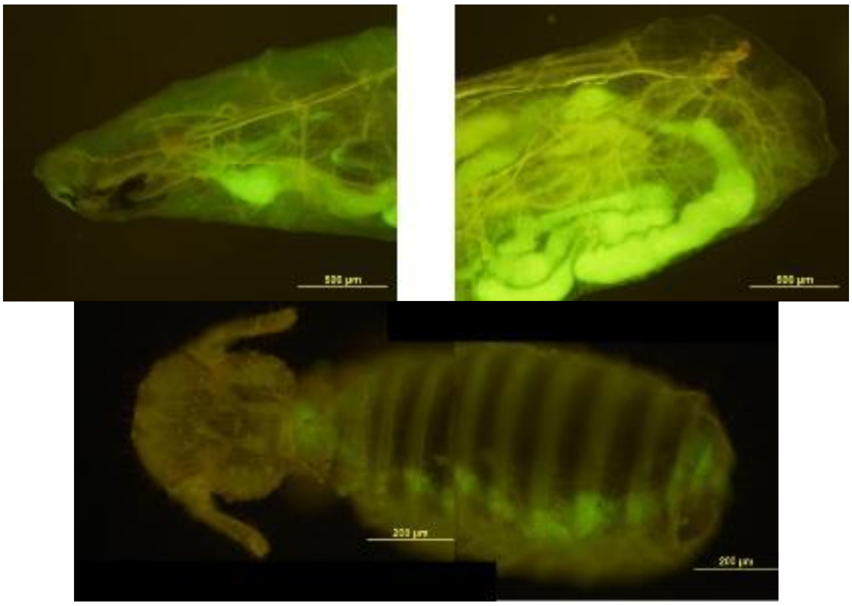

Figure 4. Fluorescein-labelled rough nanoparticles ingested during feeding in assays with first stage sheep blowfly larva (fluorescence in the anterior and posterior sections of the gut shown) and an adult sheep louse.

Testing against sheep blowflies
To test the relative efficacy of different formulations in the presence of environmental influence such as photo-degradation and leaching from the fleece by rainfall a series of laboratory tests with *L. cuprina* larvae have been conducted. Formulations for the tests were dispersed in the carrier compound (water for lipophilic pesticides, hexane for water soluble pesticides) by ultrasonication for 1 hour and applied to wool staples collected from a Merino fleece known to have had no previous chemical treatment. First stage blowfly maggots were then exposed to the treated wool using standard larval assays. To test the effects of photodegradation with the different nanoparticle formulations the treated wool samples were first exposed to ultra-violet radiation by two methods, an artificial UV exposure regime in the laboratory, or extended exposure to natural sunlight on the roof of the EcoSciences precinct in Brisbane (Figure 5).

The incorporation of water soluble chemicals may offer potential for development of a formulation that is strategically released under moist conditions, but which remains inert in the fleece when there is no moisture and therefore no flystrike risk, or which is only released in the insect gut following ingestion. That is, a formulation with a longer presence in the fleece and designed to release only at times and in sites where control is needed.

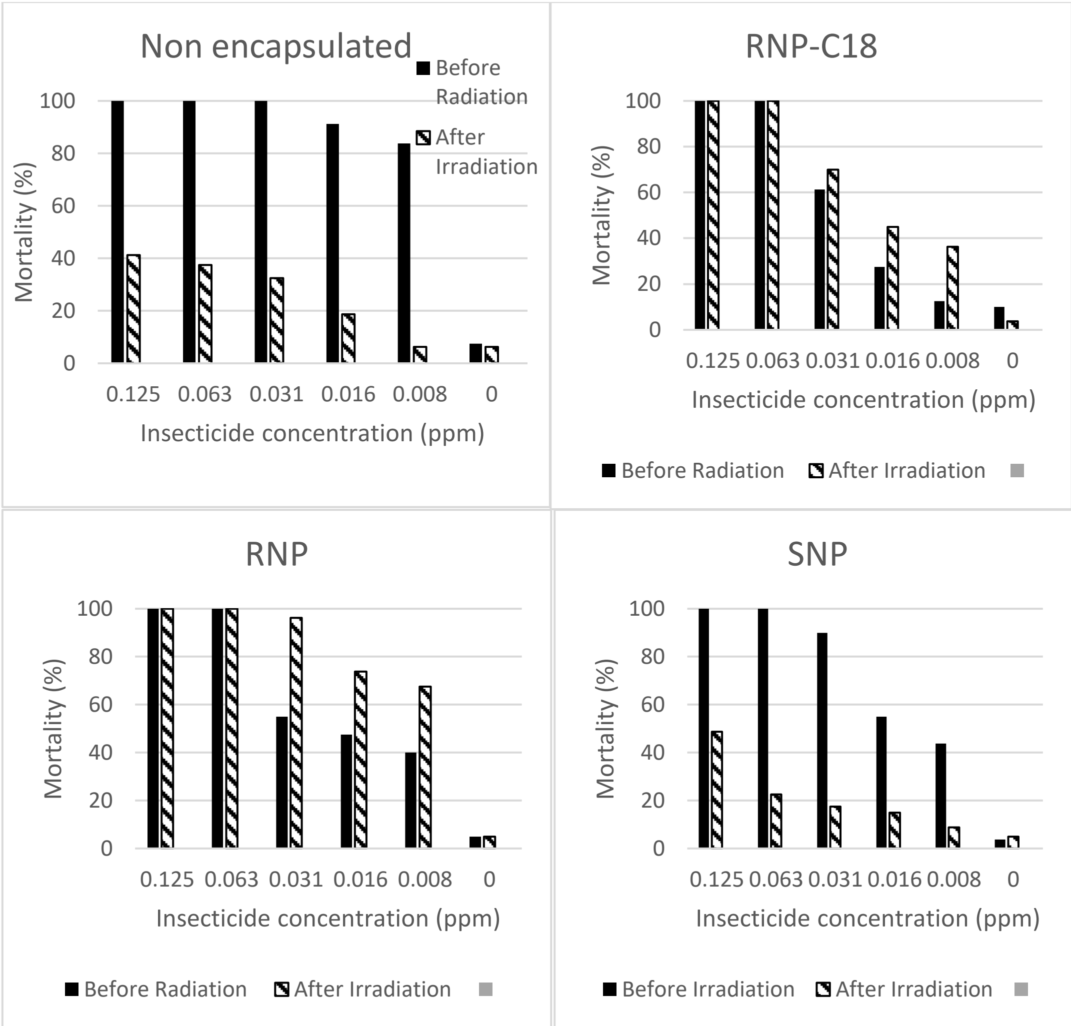

Figure 5. Effect of UV exposure on efficacy of nanoparticle formulations of lipophilic insecticide and a commercial formulation in larval assays.

Figure 5 shows the mortality of larvae exposed to wool treated with nanoparticles containing a lipophilic chemical following exposure of the wool to high-level UV radiation. As with most of the assays conducted, the rough nanoparticle formulations suffered much less degradation, and remained effective against the exposed larvae whereas the effectiveness of the unencapsulated chemical and the smooth nanoparticle formulations larvae was considerably reduced after irradiation.

Figure 6 suggests that the rough-surface particles also assist in reducing leaching of water-soluble chemical from the wool. After the wool samples had been exposed to approximately 6 cm of simulated rainfall on two occasions there was a significant decrease in efficacy of the unencapsulated chemical whereas the decrease was relatively small with the FSN-60 and RNP chemicals.

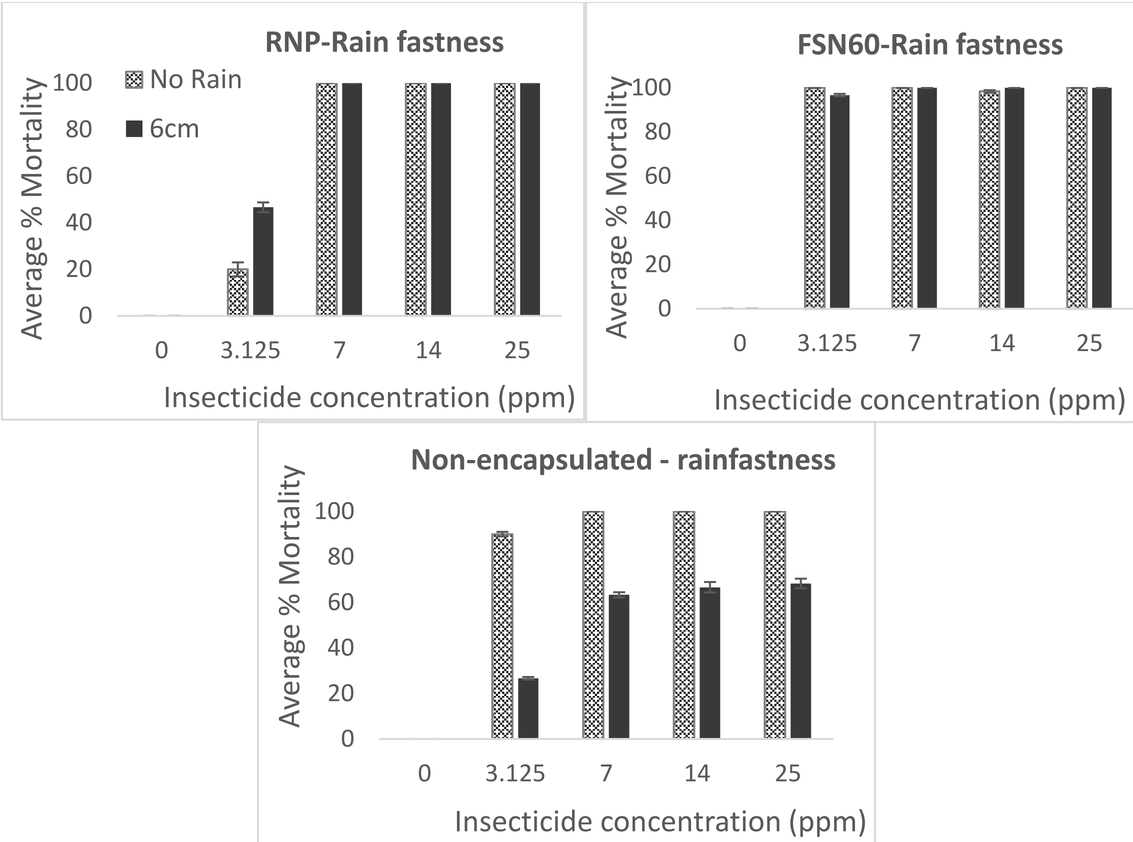

Figure 6. Larval toxicity in assays for rain fastness with wool treated with different formulations of water-soluble pesticide then exposed to simulated rainfall on two occasions.

Low residue chemicals and plant extracts

A large range of plant extracts and other chemical compounds have been shown to have insecticidal and repellent effects against *L. cuprina*. Although these compounds can often give short term protection, their effectiveness is usually rapidly lost due to volatilisation and environmental degradation. However, our results to date suggest that degradation can be significantly reduced by incorporation in rough silica nanoparticles and that appropriate formulation may be able to make their decay profile more favourable for practical use (Figure 7).

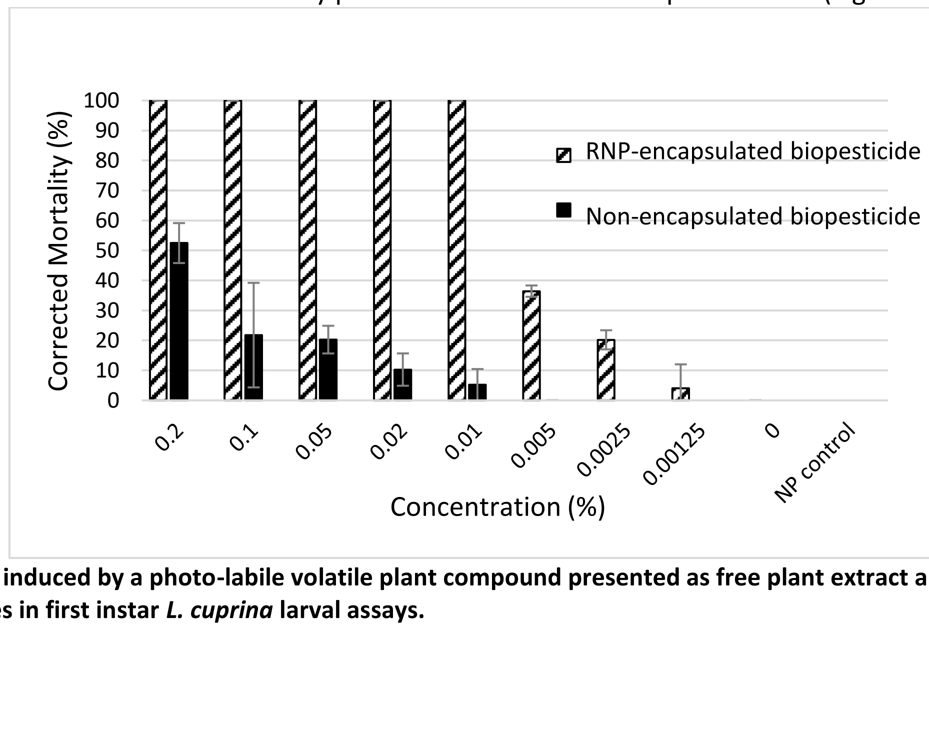

Figure 7. Mortality induced by a photo-labile volatile plant compound presented as free plant extract and encapsulated in rough nanoparticles in first instar *L. cuprina* larval assays.

CONCLUSION

Huge advances in controlled release technology for a wide range of applications, and in particular nanotechnology, offer significant opportunities for the development of new or enhanced sheep blowfly and lice control strategies. Although there have been some studies in this area in the past (Anderson et al. 1989, James et al. 1990, 1994, Rugg et al. 1998) for a range of reasons these have largely not been pursued.

The silica nanoparticles described here are environmentally degradable, have low health risk and importantly can be applied by conventional application equipment. As shown here they provided better protection in the presence of environmental challenge in laboratory tests. Studies are now required to test the behaviour of the particles in the sheep wool-skin environment to see if extended protection can be obtained from these formulations under more practical conditions.

What has long been considered the cardinal rule of toxicity, 'dose makes the poison' has been attributed to Paracelsus, a 15th century Swiss physician. This has more recently been elaborated to 'Dose makes the poison – but formulation is the key'. Nowhere would this seem to be more appropriate than with the possibilities presented by nanotechnology.

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Dr Peter James' main area of interest is in new technologies and integrated approaches to the control of pests and parasites associated with domestic livestock species. He has worked with ectoparasites and nuisance insects associated with most of Australia's major livestock industries and involvement with research and extension on sheep ectoparasites extends over more than 30 years. Formally at SARDI and the Roseworthy Campus of University of Adelaide in SA, he is currently a Senior Research Fellow at the Queensland Alliance for Agriculture and Food Innovation (QAAFI) at the University of Queensland. Peter's PhD was on the biology and population dynamics of sheep lice, he was leader of the team that developed LiceBoss, and he is a member of the ParaBoss Technical Advisory Committee.

NEW CHEMICALS FOR FLYSTRIKE CONTROL

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SUMMARY
The project aimed to address the need for new chemicals for the control of the sheep blowfly by exploring the potential of a new class of insecticides. We designed and synthesised experimental compounds and showed that they were very effective at killing blowfly larvae, both in the laboratory and on sheep, at concentrations comparable to the commercial blowfly insecticide cyromazine. Further work to build on the outcomes of the present study to develop these insecticides for blowfly control will require the project team engaging with animal health companies. Given the ability of the blowfly to develop resistance to the chemicals used for its control, it is important that new classes of insecticides, such as the type explored in the present project, are in the development pipeline to provide these future chemical control options.

PROJECT REPORT
Control of the sheep blowfly relies largely on the use of chemical insecticides applied as preventative treatments to protect against flystrike. However, recent reports of the emergence of resistance to the most commonly-used chemicals threaten the sustainability of the industry, and have highlighted the need for alternative drugs for flystrike control. The present project aimed to explore one avenue of this drug development process by examining the potential for blowfly control based on the use of inhibitors of a specific target in the blowfly. The target was a group of enzymes that play a vital role in cell development in most organisms, histone deacetylase enzymes (HDACs). In recent years there has been a great deal of interest in developing inhibitors of these enzymes in humans as possible treatments for cancers, inflammatory diseases, and parasitic diseases. The present project aimed to identify inhibitors of HDAC enzymes for use as insecticidal compounds for the control of the sheep blowfly.

Experimental HDAC inhibitors were synthesised, and their ability to kill blowfly larvae was measured using *in vitro* assays. We also measured the ability of the compounds to inhibit the blowfly HDAC enzymes. We undertook repeated rounds of compound synthesis and testing, using the results of each round to inform on structural changes to be made to compounds for the next round of synthesis. We also performed a comprehensive homology modelling study to generate likely structures of the blowfly HDAC enzymes. This then allowed us to model the fit of experimental drugs into the enzymes. The homology modelling also allowed us to study differences that exist between the structures of the enzymes in blowflies and mammals, with a view to exploring the potential for drug design of blowfly-specific inhibitors. Finally, to begin to translate our study from the lab to the field, we conducted a small scale larval-implant trial on sheep using several of our experimental compounds. We examined the ability of blowfly larvae to establish strikes on sheep at sites that have been treated with the experimental drugs.

The most potent compounds identified in the study had very significant levels of activity against blowfly larvae *in vitro*, and were also potent inhibitors of blowfly HDAC enzymes. The best of the compounds was within 4-fold as toxic to blowfly larvae as the commercial blowfly control chemical cyromazine (the active ingredient in Vetrazin, ProGuard, Lucifly and Cy-Guard) in our *in vitro* assays. Importantly, the most potent compounds showed an ability to inhibit the early larval life stages of the blowfly, with complete inhibition of larval growth within the first 24 hours at the highest concentrations tested. This speed of action of the compounds is an important aspect for their potential as insecticides as it is vital for a blowfly control chemical to prevent the larvae developing to a stage that can start to cause significant damage to the sheep.

We constructed *in-silico* homology models for each of the five blowfly HDAC proteins *Lc*HDAC1, 3, 4, 6 and 11, as identified in its genome. The various blowfly HDACs had between 44 -78% sequence identity with their respective human HDACs (1, 3, 4, 6, 11). We analysed the amino acid differences between the blowfly HDACs and their corresponding human HDACs near the binding site. We found the binding sites of three of the blowfly enzymes were very similar to human binding sites with few differences. Hence, the design of inhibitors that are selective for these blowfly enzymes over their human counterparts will be challenging. On the other hand, for another of the blowfly enzymes (*Lc*HDAC6), we found significant sequence differences between the human and blowfly binding sites. Drug docking studies confirmed the presence of a number of differences near the binding site of the human and blowfly HDAC6 enzymes. These differences in residue size, charge and polarity may allow the design of new inhibitors that may prove to be more potent and selective towards blowfly HDACs.

Finally, we conducted a sheep trial in which experimental compounds were applied to sites on sheep, and the subsequent ability of blowfly larvae to establish infections at these sites was measured (all animal procedures were approved by the CSIRO Armidale Animal Ethics Committee, approval number 19/04). We applied cyromazine (ProGuard) to some sites as a commercial insecticide treatment to compare to our experimental compounds. We tested three experimental inhibitors, chosen on the basis of potency against blowfly larvae *in vitro*, high microsomal metabolic stability, presence of structurally-distinct features, and low synthetic cost. Two of the three compounds killed all the larvae at the experimental sites. The level of drug required to kill all larvae was approximately 5-fold higher than the levels of cyromazine required to achieve the same outcome. This indicates that the experimental compounds were able to prevent blowfly larval growth at a concentration similar to that for the commercial product cyromazine.

Figure 1. Measuring the ability of experimental compounds to prevent growth of blowfly larvae on sheep. Left: experimental sites on a sheep; Right: addition of freshly-hatched blowfly larvae on a paper disc to a drug-treated experimental site.

We investigated whether any of the experimental drugs were blowfly-specific inhibitors (that is, inhibitors of blowfly HDAC enzymes, but not of mammalian HDAC enzymes), but found no evidence for this. However, as mentioned above, the homology modelling work showed that a focus on the *Lc*HDAC6 enzyme offers potential for the discovery of such blowfly-specific inhibitors in the future. It is also clear that complete insect-specificity may not be required for blowfly control as the potency of the experimental compounds identified here means that they can likely be used at levels safe for topical application to mammals, as required for blowfly control in sheep.

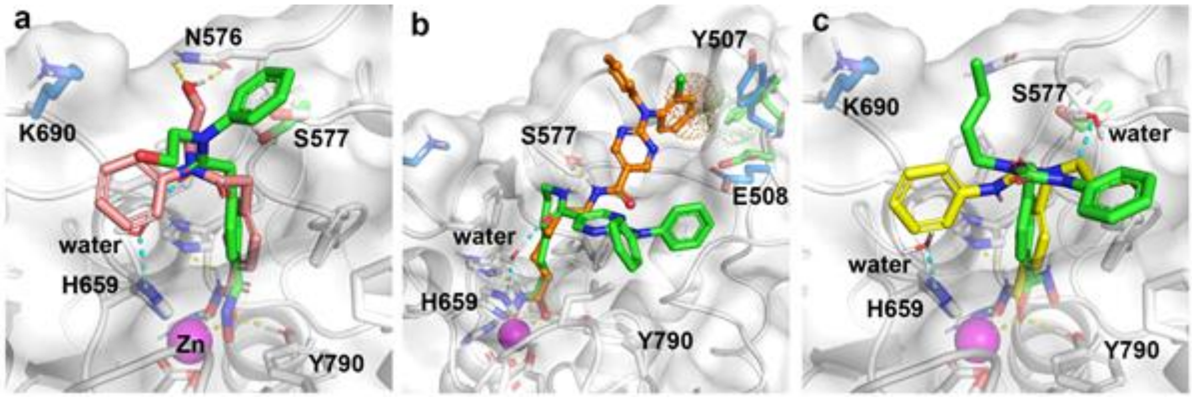


Figure 2. Modelling of blowfly HDAC enzymes to determine how well experimental compounds 'fit' into their target enzyme in the blowfly.

This project has shown that HDAC inhibitors are potent inhibitors of blowfly larval growth and development *in vitro* and has identified new potent compounds. We have shown that the most potent compounds are also able to prevent the development of larvae at experimental sites on sheep. The project represents the first stage of a process in drug development that can take many years. It would be at least 5 years before any insecticide based on inhibition of HDAC enzymes could be delivered to the market, perhaps longer. In the meantime, the wool industry is able to utilise the currently available insecticides, with the knowledge however that resistance to the dicyclanil-based products is emerging. If resistance to this chemical becomes more wide-spread, and resistance also emerges to the currently-used ivermectin- and imidacloprid-based products, the availability of new insecticides will become more important and their rapid development more urgent. It is therefore important that new classes of insecticides, such as the type explored in the present project, are in the development pipeline to provide these future chemical control options.

Further work to build on the outcomes of the present study to develop HDAC inhibitors as insecticides for blowfly control will require the project team engaging with animal health companies.

FURTHER INFORMATION

A final report on this project is available at www.wool.com/flystrikelatest.

Dr Andrew Kotze is a parasitologist with CSIRO Agriculture and Food. He is also an Adjunct Professor at the University of Queensland and Editor-in-Chief of The International Journal for Parasitology: Drugs and Drug Resistance. His research interests are in chemical control of insect and nematode parasites of sheep, cattle, companion animals and humans. He also studies the mechanisms of drug resistance in these parasites.

Professor David Fairlie is an NHMRC Senior Principal Research Fellow in the Institute of Molecular Bioscience at the University of Queensland. He leads a multidisciplinary group of around thirty senior research scientists, postdoctoral fellows and graduate students at interfaces between chemistry and biology and tackles interdisciplinary problems, in science, health and industry, including in the development of new experimental medicines His focus is on understanding mechanisms of chemical and biological processes, disease development and drug action.

HOW TECHNOLOGY CAN HELP

AUTHOR
Dr Carolina Diaz, AWI Program Manager Agri-Technology

HOW TECHNOLOGY CAN HELP?
Automation will bring efficiencies in farm data collection, decision-making and labour saving. Using sensing technologies, artificial intelligence or robotics, woolgrowers will be able to optimize their production systems and to be more profitable, efficient, safer, and more environmentally friendly.

These technologies will also contribute to improved animal welfare and reducing disease incidence, increasing the monitoring and control of the animals and facilitating early detection and prevention.

Several AWI investments in Agri technology are investigating the direct or indirect detection, control or reduction of the incidence of flystrike in the flock. These projects are summarised below.

AWI SMART TAGS

AWI's smart tags aim to enable woolgrowers to track, monitor and assess the status of their flock in real time and make more informed decisions to increase their enterprise's profitability.

The ultimate goal is to provide a tool to remotely monitor the animals, detect welfare issues, improve reproduction management and collect data from the paddock. AWI anticipates that smart tags could be used for research and data analysis purposes to help woolgrowers optimise their flock's productivity. For example, in relation to flystrike management, wild dog alerts, grazing optimisation, health alerts and reproduction optimisation.

AWI is collaborating with various research organisations to investigate different behaviours and relating them to specific paddock events or welfare issues. Some of the objectives are:

- IMPROVING REPRODUCTIVE MANAGEMENT: In collaboration with the University of Sydney, this project aims to understand male and female reproductive behaviour such as heat, joining or lambing and how these behaviours can be detected using AWI smart tags.

- IMPROVING GRAZING MANAGEMENT: Murdoch University, DEDJTR (Victoria) and NextGen Agri will extend the capacity of the AWI smart tags to enable woolgrowers to optimise grazing decisions. The project will generate smart tag data across a range of grazing situations and use this data to train machine learning algorithms that can accurately predict feed on offer and detect grazing behaviour which, for example, will inform decisions regarding the time to shift sheep between paddocks or determine the appropriate amount and timing of supplementation.

- PREDATION AND WELFARE ALERTS: Central Queensland University researchers are assessing the ability of AWI smart tags to help woolgrowers detect animal health and wellbeing issues faced by sheep. Predation by wild dogs and animal health issues (worms, flies, lice) are major issues for the wool industry with significant impacts on profitability and animal welfare. AWI smart tags have the potential to provide woolgrowers with an early warning of behaviours associated with predation by wild dogs or the development of more subtle welfare issues in individual sheep in their flock. This will enable producers to detect and manage problems well before they turn into more significant issues.

ARTIFICIAL INTELLIGENCE IN WOOL PRODUCTION

Favourable results were achieved in an AWI-funded pilot study led by NextGen Agri and University of Sydney. The project provided a pilot evaluation on the utility of Artificial Intelligence (AI), and in particular, Deep Learning, in accurately predicting performance outcomes from images, biomarkers and on-animal sensor output.
This project clearly demonstrated that with the correct training data set, machine learning models will be very powerful in predicting a range of informative traits from image-based inputs of sheep. This could be used for example to predict breech strike susceptibility based on image data, to facilitate data collection in the paddock and to normalize scoring systems.

The researchers also evaluated potential scenarios for AI to be used in complex decision-making processes and combining complex data from multiple sources which again, could represent an opportunity for these technologies to support flystrike management strategies.

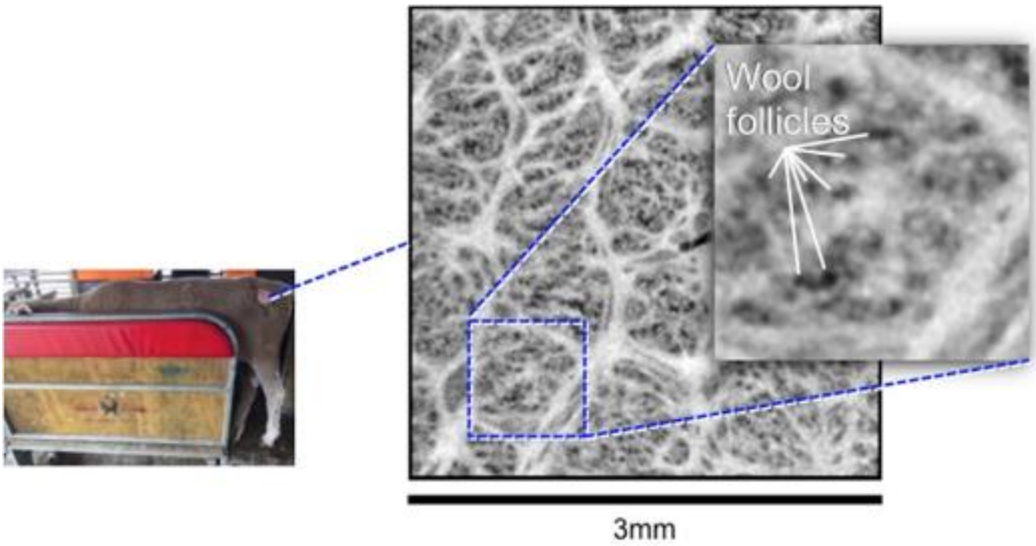
FOLLICLE DENSITY MEASUREMENT AND DIAMETER

There are good prospects for reducing the incidence of flystrike over the longer term by breeding for key flystrike resistant traits, i.e. reducing breech wrinkle, breech wool cover, dags and urine stain.

Breeding for lower wrinkle may lead to decreased fleece weights although sheep that are relatively high in fleece weight and low in wrinkle have been identified, and the industry is selecting and breeding from these to ensure they maintain and/or increase productivity whilst decreasing wrinkle in their flocks.

However, by selecting sheep with an optimum density of wool fibres we can improve both quantity and quality. In addition, wool follicle density is highly heritable so genetic gains can be made if the trait can be measured cost effectively.

This project led by the University of Adelaide aims to develop a point-and-click scanner that enables farmers to measure wool follicle density and diameter on-farm and with sheep at a much younger age than is currently possible at a much lower cost than presently available. Safe, non-invasive imaging can look below the skin surface and analyse the wool follicles. This will allow woolgrowers and ram breeders to identify high value sheep earlier, which could increase productivity of current sheep flocks and facilitate increased rate of genetic improvement.



Picture: University of Adelaide

VALIDATION OF PULSE OXIMETERS

Could pulse oximeter technology detect changes in blood Oxygen levels that that may coincide with early signs of *Haemonchus contortus* (Barber's Pole Worm) infections in sheep?

AWI is collaborating with Dawbuts in this proof of concept study that may provide the foundation for further research into practical on-farm devices such as ear tags with built-in pulse oximeter sensors.

Ideally, these would allow for continual monitoring and provide early detection signals to farm managers, such that *H. contortus* infections can be treated before they compromise animal health and welfare.

MORE INFORMATION

More information on the AWI Agri-Technology program is available at: https://www.wool.com/sheep/agritechnology/ or contact Carolina Diaz email: carolina.diaz@wool.com.

Dr Carolina Diaz is the Program Manager for Agri-Technology at Australian Wool Innovation. After some time working as a veterinary practitioner in Spain, she started to work for the Spanish government in national and European projects related to improvement and conservation of indigenous breeds, traceability and animal identification.

Carolina moved to Australia with her family three years ago and joined AWI, where she has kept nurturing her passion for innovation and Agri-tech. Data activist and firmly convinced that technology has come to Agriculture to stay.

RATE OF GENETIC GAIN IN REDUCING BREECH FLYSTRIKE

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SUMMARY
Genetic gains were predicted for reducing breech flystrike incidence (FSI) based on selection using modified MERINOSELECT indexes. Predicted genetic reductions in flystrike incidence after 10 years of selection were larger when the heritability of the trait was moderate (as in a summer rainfall environment) than when a low heritability was assumed (as in a winter rainfall environment). The relative gains between flystrike incidence and fleece weight, fibre diameter and reproductive rate and their implications for Merino breeding programs are discussed.

INTRODUCTION
General consensus within the Australian wool industry is that breeding more resistant sheep will play a critical role in controlling breech and tail strike in non-mulesed flocks as well as reducing the risk of strike in mulesed sheep. Since 2005, a major research program has been funded to identify optimal breeding strategies for reducing breech strike resistance. Selection lines for breech flystrike were established at Mt Barker in WA, in a winter rainfall environment (Greeff and Karlsson 2009) and near Armidale, NSW, in a summer rainfall environment (Smith *et al.* 2009). Results have confirmed the presence of significant genetic variability amongst sheep in susceptibility to breech strike and also identified key indirect selection criteria for improving resistance, in particular scores of breech wrinkle, dag, breech cover and urine stain (Smith *et al.* 2009; Greeff *et al.* 2014). Earlier studies (Brown *et al.* 2010; Richards and Atkins 2010) predicted genetic gains from index selection with breech wrinkle score included in the breeding objective, as a proxy for breech flystrike incidence. This project extends earlier findings, by predicting genetic gains in breech flystrike incidence from selection using all available indicator traits of breech wrinkle, dag and breech cover scores for three different breech objectives within three different environments.

METHODOLOGY
Predictions of genetic gain from within flock selection were undertaken based on software written by members of AGBU staff. Three breeding objectives were examined, by modifying the Dual Purpose (DP+), Fibre Production (FP+) and Merino Production (MP+) indexes available from MERINOSELECT (Sheep Genetics 2018). The modifications consisted of adding Flystrike incidence (FSI, strikes/ewe/year) as a formal trait to the breeding objective associated with each index. The modified indexes, DP+FSI, FP+FSI and MP+FSI target, respectively, medium wool/dual purpose, superfine/fine wool and fine/medium wool production systems, along with reduction in flystrike incidence. The effects of using a range of flystrike incidence economic values were examined, from $0 to -$240/strike/ewe/year, to include the likely large range in the associated costs of flystrike incidence across Australia. Genetic parameters used in predictions were from Sheep Genetics, supplemented by values from Hatcher and Preston (2017, 2018) and AWI project reports (Greeff *et al.* 2016; Smith 2016). Full records of productivity traits and pedigrees were assumed as selection criteria, as well as records of breech wrinkle, dag and breech cover scores. Predictions were conducted for 3 different scenarios (i) moderate heritability (0.20) for (ii) low heritability for flystrike incidence (0.10) and (iii) low heritability for flystrike incidence (0.10) but high heritability (0.30) for Dag Score.

RESULTS AND DISCUSSION

Genetic gains in flystrike incidence (Figure 1). After 10 years of index selection, with moderate heritability (scenario i), predicted gains for flystrike incidence range from a low of -0.02 to -0.03 (2% to 3%) to -0.19 to -0.21 (19% to 21%) strikes/ewe/year, when the economic value is increased from 0 to -$240. These predicted gains are 3 to 5 times as large as those where heritability for flystrike incidence is low (scenario ii). Gains are slightly less for the FP+FSI index and slightly more for the MP+FSI index compared to gains from using the DP+FSI index. At the highest economic value, most (80%+) selection emphasis is placed on reducing flystrike incidence (not shown). Predicted gains for scenario iii are no different to scenario ii, so are not discussed further.

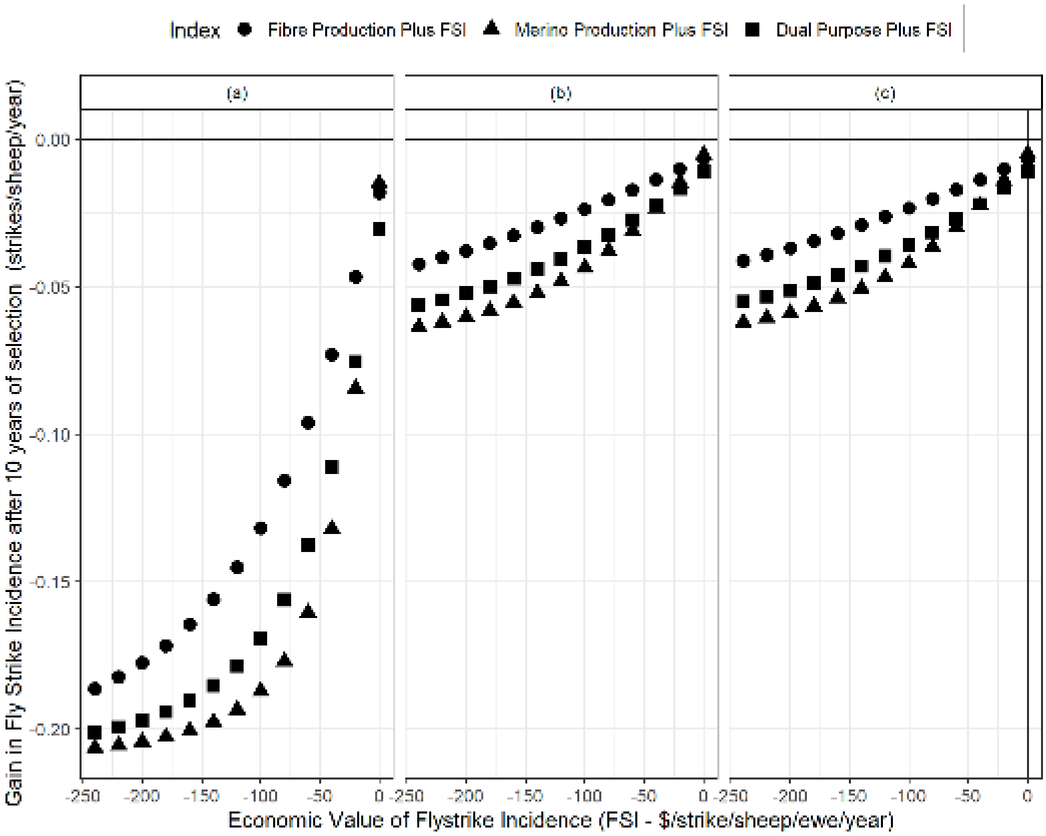

Figure 1. Predicted 10-year genetic gains in Flystrike Incidence (FSI, strikes/ewe/year) assuming (a) moderate FSI heritability (b) low FSI heritability and (c) low FSI heritability and high Dag Score heritability, with economic values from 0 to -$240 for FSI.

When compared to breech flystrike incidence in unmulesed, crutched young sheep in a winter rainfall environment in WA (see Greeff *et al.* 2016) and in a summer rainfall environment near Armidale, NSW (Smith 2016), our predicted genetic gains suggest that once a flock was fully pedigreed and all important breech and productivity records were being collected, after 10-15 years of index selection, flystrike incidence could be reduced in unmulesed sheep to low levels (<0.02 or 2%) in average years. It is however important to balance potential gains with the predicted impact on genetic gains for other important traits, shown below.

Genetic gains in key production traits. Predicted genetic gains for Clean Fleece Weight (CFW), Fibre Diameter (FD) and the Number of Lambs Weaned/Ewe Joined (NLW) are listed in Tables 1 to 3, respectively, listed by economic value for flystrike incidence. With increasing economic value for flystrike incidence, gains in Clean Fleece Weight decrease gradually when the heritability of flystrike incidence is low, but decrease more rapidly when the heritability of flystrike incidence is moderate, particularly for the DP+FSI and MP+FSI indexes. However, even for the largest economic value for flystrike incidence examined (-$240), genetic gains for fleece weight remain positive.

Table 1. Predicted genetic gain in Clean Fleece Weight (%) after 10 years of index selection, by economic value for Flystrike Incidence (FSI)

h^2 FSI/Index	Economic Value for FSI ($/strike/ewe/year)						
	0	-40	-80	-120	-160	-200	-240
Low h^2							
DP+FSI	2.69	2.50	2.27	2.03	1.80	1.59	1.39
FP+FSI	2.69	2.59	2.48	2.30	2.20	2.06	1.92
MP+FSI	6.04	5.58	4.92	4.22	3.59	3.06	2.63
Moderate h^2							
DP+FSI	2.69	2.07	1.46	1.01	0.70	0.48	0.32
FP+FSI	2.69	2.37	1.97	1.59	1.26	1.00	0.80
MP+FSI	6.04	4.34	2.77	1.84	1.29	0.92	0.67

Table 2. Predicted genetic gain in Fibre Diameter (μ) after 10 years of index selection, by economic value for Flystrike Incidence (FSI)

h^2 FSI/Index	Economic Value for FSI ($/strike/ewe/year)						
	0	-40	-80	-120	-160	-200	-240
Low h^2							
DP+FSI	0.01	-0.03	-0.07	-0.11	-0.14	-0.16	-0.18
FP+FSI	-0.78	-0.80	-0.81	-0.81	-0.81	-0.80	-0.79
MP+FSI	-0.36	-0.42	-0.44	-0.45	-0.44	-0.44	-0.43
Moderate h^2							
DP+FSI	0.01	-0.10	-0.17	-0.21	-0.23	-0.25	-0.26
FP+FSI	-0.78	-0.81	-0.79	-0.75	-0.70	-0.66	-0.62
MP+FSI	-0.36	-0.45	-0.43	-0.40	-0.38	-0.37	-0.36

When the heritability of flystrike incidence is moderate, there are small genetic reductions predicted in Fibre Diameter with increasing flystrike incidence economic value using the DP+FSI index of up to 0.26μ after 10 years of selection. There are also slightly lower genetic gains in Fibre Diameter with increasing flystrike incidence economic value using the FP+FSI index of up to 0.16μ. However, in general there is little impact predicted on genetic gains for Fibre Diameter when also selecting for reduced flystrike incidence as part of index selection. Predicted genetic gains for the Number of Lambs Weaned when also selecting for reduced flystrike incidence are impacted much less than those for fleece weight. Relative to gains when flystrike incidence has no economic value, there are modest declines of up to a maximum of 31% in the Number of Lambs Weaned gains using the MP+FSI index and up to 45% using the DP+FSI, at the largest economic value for flystrike incidence examined (when h^2 for FSI is moderate).

Table 3. Predicted genetic gain in the Number of Lambs Weaned/ewe after 10 years of index selection, listed by economic value for Flystrike Incidence (FSI)

h^2 FSI/Index	Economic Value for FSI ($/strike/ewe/year)						
	0	-40	-80	-120	-160	-200	-240
Low h^2							
DP+FSI	0.08	0.08	0.08	0.07	0.07	0.07	0.06
FP+FSI	0.02	0.02	0.02	0.02	0.03	0.03	0.03
MP+FSI	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Moderate h^2							
DP+FSI	0.08	0.07	0.07	0.06	0.05	0.05	0.04
FP+FSI	0.02	0.02	0.03	0.03	0.03	0.03	0.03
MP+FSI	0.04	0.04	0.04	0.03	0.03	0.03	0.03

CONCLUSIONS / KEY MESSAGES

Useful and meaningful reductions in breech flystrike from 0.05 to 0.08 strikes/ewe/year up to 0.19 to 0.21 strikes/ewe/year in a summer rainfall low dag environment were predicted after 10 years of index selection for breeding objectives relevant to the Australian wool industry (once full pedigree and data recording has commenced). For a winter rainfall environment in WA, with a lower heritability for flystrike incidence, smaller gains were predicted after 10 years of index selection, from 0.01 to 0.02 up to 0.04 to 0.06 strikes/ewe/year.

The length of time required to breed sheep that no longer require mulesing not only depends on the rate of genetic gain that can be achieved, but also on how susceptible the sheep are to breech flystrike at the start of the breeding program and the site's relative risk of flystrike. For example, sheep types with high wrinkle scores and / or high dag scores will take considerably longer to reduce susceptibility to breech flystrike to a point where mulesing could be stopped.

It is unnecessary to use very high economic values for flystrike incidence to achieve significant reductions in the trait. For example, when the heritability for flystrike incidence is moderate and the trait is given an economic value of -$80, 62% of the highest genetic gain for flystrike incidence predicted (when the economic value is -$240) is obtained with the FP+FSI index, with higher gains of 78% and 86% being obtained with the DP+FSI and MP+FSI indexes, respectively. At that economic value for flystrike incidence (-$80), between 46% to 73% of the genetic gains for fleece weight and between 84% and 125% of the genetic gain for the Number of Lambs Weaned are being retained, with no significant impact on gains in Fibre Diameter. In the absence of formally-derived economic values, -$80 for flystrike incidence appears to be a reasonable upper limit to use in Merino breeding programs to achieve a balance between genetically lowering flystrike incidence whilst obtaining competitive genetic gains in productivity traits as outlined above.

The take home messages are:

- Meaningful reductions in flystrike incidence are possible in ram breeding flocks over a 10 to 15-year period after full data recording has commenced, whilst retaining competitive levels of genetic gains for other important traits, by using appropriately constructed selection indexes. This amount of genetic gain could reduce breech flystrike incidence sufficiently to reduce reliance on mulesing, or to cease it.
- With the aid of appropriately weighted selection indexes, breeders do not actually have to accept going backwards in genetic merit for any important trait when incorporating reducing flystrike incidence in their breeding objectives, but there will be a reduction of rates of genetic gain that can be made for some traits, in particular fleece weight, with gains reducing by 27% up to 50%.

RECOMMENDATIONS

- Estimate economic values for flystrike incidence for different wool-growing regions for establishing formal breeding objectives that include reducing flystrike incidence along with productivity and quality traits.
- Develop new selection indexes that incorporate animal welfare / resilience traits, including flystrike incidence as part of index options by the MERINOSELECT service, plus inclusion of flystrike incidence as a reportable trait.
- Active encouragement (extension and promotion) to industry to increase the number of sheep that are recorded for breech traits and for neck and body wrinkle if they have already been mulesed or are already very low for breech wrinkle.
- Explore the merit of direct progeny testing of leading industry sires for flystrike incidence, particularly for areas of high dag incidence. This should be done in conjunction with establishing a reference population for the development of genomic enhanced breeding values.
- If ram buyers are having difficulty accessing suitable flock ram genetics to more rapidly reduce breech flystrike incidence and keep improving flock productivity, establishing their own ram breeding nucleus and purchasing semen from elite sires may be more economically feasible for their particular breeding objectives, management regime and locality.
- Set target ASBVs to go non-mulesing.

FURTHER INFORMATION

An article communicating project outcomes to woolgrowers was published in the June 2019 edition of *Beyond the Bale*. The article and the final project report are both available at www.wool.com/flystrikelatest**,** or at the links below.

- Project Report – Rate of Genetic Gain in Reducing Breech Flystrike - Update June 2019
- Genetically Reducing Breech Flystrike – June 2019 *Beyond the Bale* article

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Forbes has spent all of his career working with the Australian sheep and wool industries, in research, technology transfer, research management and also as a commercial wool producer. He has specialized in applied genetics and has been involved with AWI's breech strike R, D and E program as a reviewer since 2010.

GENOTYPING OF BREECH FLYSTRIKE RESOURCE FLOCK

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SUMMARY
Breech flystrike is a costly trait to measure. Australian Wool Innovation's investment into genetic solutions for breech flystrike has resulted in the availability of estimated breeding values for indicator traits, such as breech wrinkle, breech cover and dag through SheepGenetics to enable genetic and permanent improvement in breech flystrike through selection. However, because selection is still based on indicator traits rather than breech flystrike itself, genetic gains are not at their maximum yet. Genomic information provides approaches that could help fast-track genetic gains in the trait of breech flystrike resistance itself.

The data and materials collected on the Breech Flystrike Resource flocks in NSW and WA form an exceptional and unique resource to explore genomic approaches. The data set in this study forms the largest reference population for breech flystrike resistance to date. It was investigated whether genomic approaches based on genes of major influence exist and enable approaches such as marker assisted selection, whether genomic selection based on genomic breeding value estimated from all available genomic information or whether a combination of the two would be the most efficient application of genomic information for selection for breech flystrike resistance.

The project demonstrated that genomic selection based on genomic breeding values will be the most efficient approach to create benefit to industry within the next 5 years. Based on the approaches evaluated in this project it is concluded that the existing genomic breech flystrike data resource provides a basis for industry to create reliable selection tools that provide the fastest genetic progress in five years' time.

PROJECT REPORT

DATA

The data comprised ~1,500 animals of the WA and NSW Breech Flystrike Resource flocks that had phenotypes and genomic information on 600K genetic DNA markers available. Traits analysed included breech flystrike (STRIKE), coded as "struck" or "not struck", and breech cover (BCOV), dag score (DAG) and wrinkle score (WRK), all categorised as low, medium or high. A genome wide association study was conducted for each of the traits to establish if any single or small number of markers could be detected with major effect on any of the traits. In addition, genomic breeding values were estimated using the information of all genetic markers.

RESULTS AND DISCUSSION

Genomic analyses point at the most efficient approaches for the application of genomic information in breeding programs. These include methods that are based on major genes, such as marker assisted selection, or based on genomic breeding values that draw on the information of all genetic markers.

As a first step it was explored if genetic markers with significant associations with STRIKE, BCOV, WRK or DAG exist. Overall, none of the genetic markers showed a large enough effect on any of the traits to be useful for marker assisted selection approaches. Although two regions were in proximity to interesting genes for STRIKE and DAG, they were not large enough to indicate that the region would harbour a gene with major effects on these traits. Further investigation might be warranted because it is possible that the data structure masks major genes. Difference in phenotype and genetic background between NSW and WA might warrant splitting the data by site or choosing extreme animals (e.g. based on lifetime strike) for potential further analyses. The results of this analysis did not support marker assisted selection as an approach for enhanced genetic gains for breech strike resistance.

The current genomic resource of the Breech Flystrike Resource flocks is an excellent start for a reference population and provided the data for the estimation of genomic breeding values. The project demonstrated that genomic breeding values can be estimated, but the current accuracy is not sufficient yet to select animals reliably for breech strike resistance. However, higher accuracies can be achieved with an increase of the number of animals with phenotypes and genotypes in the reference population. In order to build on the current reference population other aspects, have to be taken into consideration. Breech flystrike remains expensive to measure, therefore it would be costly and impractical to run a specific reference population for this trait, although, the resource could be used for genomic predictions of other traits as well. A dispersed reference population, that includes data from various sources, would be advantageous based on opportunistic sampling in flocks of key breeders, Merino Lifetime Productivity flocks, sire evaluation flocks - any flock with reliable records. However, in order to include phenotypes from different sources in a reference population, the effect of different management procedures on breech flystrike needs to be established, e.g. does breech flystrike in sheep that have been chemically treated for flystrike, correlate well with flystrike in untreated sheep. Other options of adding more phenotypes could be explored, such as the collection of “pooled” phenotypes from commercial wool production operations.

It is recommended that a future reference population should have at least 7,500 phenotypes to achieve sufficient accuracy, which would require the collection of a further 6,000 phenotypes in addition to the Breech Flystrike resource. It is suggested that a time frame of 5 years would a maximum time frame to build a dispersed reference population.

CONCLUSIONS AND RECOMMENDATIONS

Genomic selection can provide substantial benefit to wool focussed Merino breeding programs. The project demonstrated that the Breech Flystrike Resource Flock from WA and NSW are a great resource that will have impact on the wool industry in the short and long term. In this study genomic breeding values were estimated for breech flystrike resistance but they do not yet have the accuracy to be used as reliable selection tools in industry. The addition of the genomic data of the Breech Flystrike Resource flocks to the current genomic resource will provide an increase in accuracy for existing genomic breeding values for wool production traits in the short term and for breech strike resistance in the long-term.

Within the next five years, a much larger dispersed reference population could be built that increases the accuracy of genomic breeding values to a level that allows accurate selection directly on breech flystrike resistance and fast-track genetic improvement for this trait.

For a detailed description of the outcomes from this project, please see the project final report, available at www.wool.com/flystrikelatest.

The livestock industries are very high-tech these days. Genomic resources coupled with innovative measurement technologies provide large amounts of information that can be used to ensure our livestock industries remain efficient and sustainable. Dr Sonja Dominik is dedicated to harnessing the big data and to creating tools for application in the livestock breeders. Sonja has been working since 2002 with CSIRO Agriculture and Food in breeding program design for livestock and aquaculture species with a focus on integrating novel genomic technologies and characteristics, such as methane emission in sheep and immune competence in cattle.

Sonja holds a PhD in Animal Genetics and Breeding (2001) with the University of New England (UNE) in Armidale, and a Bachelor in Agricultural Sciences (Honours) with the Justus-Liebig University, Giessen, Germany (1996). Sonja has produced over 50 scientific publications and major industry reports. Sonja is passionate about engaging with industry the general community to exchange about anything related to animal breeding and the livestock industries.

BREEDING AND SELECTION – INDUSTRY TRENDS

AUTHOR
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SUMMARY
This update on sheep breeding and selection for flystrike resistance reports on changing genetic trends as indicated by MERINOSELECT ASBVs since 2000. Overall, the genetic trends for lower breech wrinkle are occurring at a modest rate that varies between the three Merino Types, Superfine, Fine and Medium wool Merinos.

However, there are individual breeders specifically breeding Merinos for low wrinkle, low dag and high productivity and these breeders are making considerable gains. The gains are easiest in medium wool Merinos in low dag country and quite difficult in Fine and Superfine Merinos in high dag country. There are now sires that are in the top 1% for low breech wrinkle, top 1% for Adult Clean Fleece Weight and top 1% on Index and also reasonable numbers of sires in the top 5% for these traits although they all tend to be high in fibre diameter. It will take time to breed flock rams with these attributes.

Evidence of the level of productivity of naturally flystrike resistant Merinos will be demonstrated in the Merino Lifetime Productivity (MLP) Project. The lifetime productivity will be determined for the progeny of 134 sires that are collectively high and low for breech wrinkle, dags, breech cover, fleece weight, reproduction, fat and muscle along with the comparison of the accuracy of early in life productivity indicators versus actual lifetime productivity. Woolgrowers will be able to compare the profitability of existing naturally flystrike resistance rams. The MLP project will answer questions and concerns that ram breeders and buyers have about the consequences of selecting for flystrike resistance and other welfare related traits (i.e. survival, worm egg count and fat).

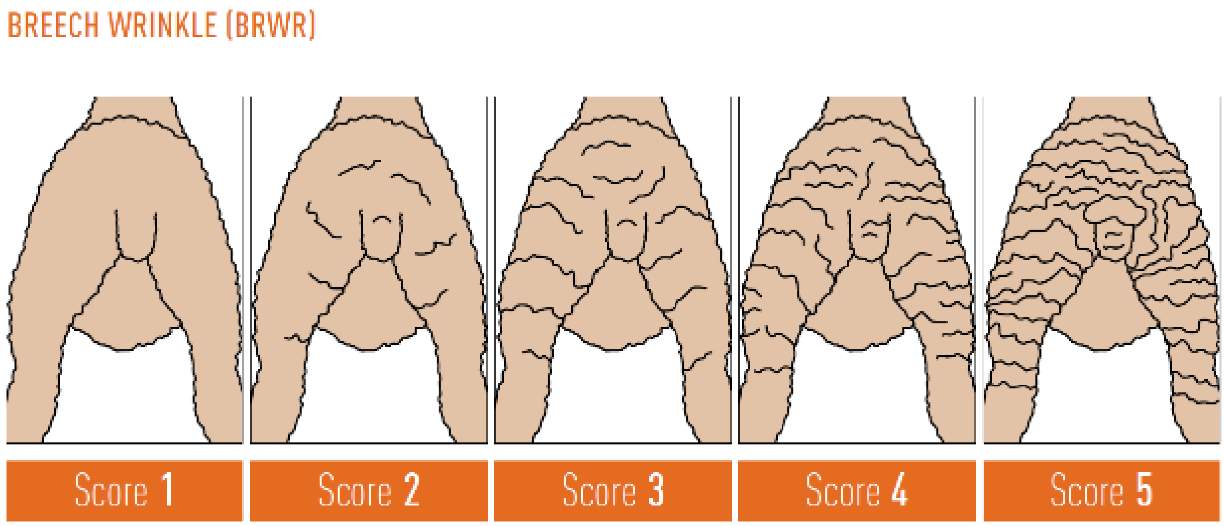

Figure 1. Visual scores for Breech Wrinkle (AWI/MLA Visual Sheep Score Guide 2019)

DAG (DAG)

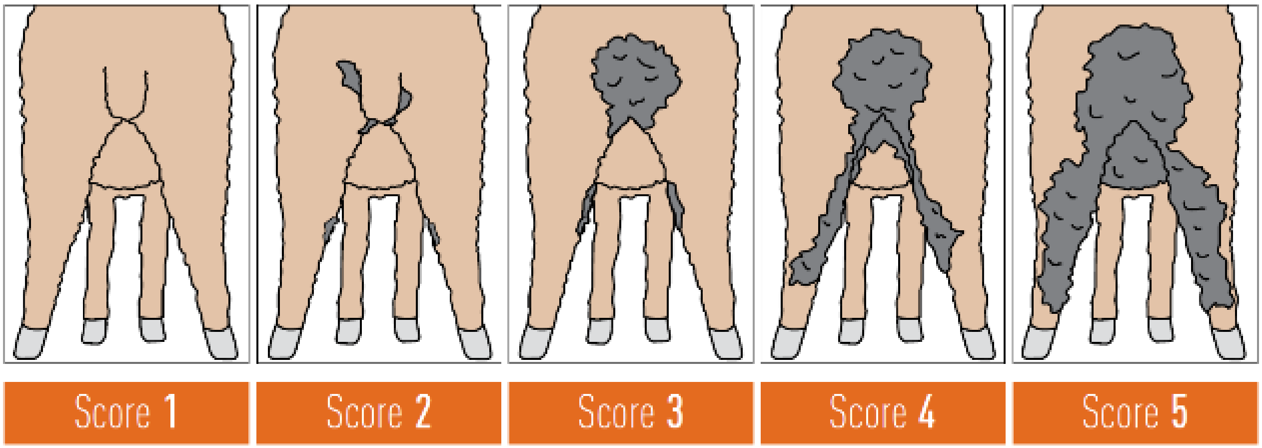

Figure 2. Visual scores for Dag (AWI/MLA Visual Sheep Score Guide 2019)

1. MERINOSELECT ASBV TRENDS SINCE YEAR 2000

Australian Sheep Breeding Values (ASBVs) are an estimate of an animal's breeding value based on its own recorded data and that of the animals in its pedigree. They allow wool growers to make a projection of how an animal's progeny will perform over a range of traits relative to other animals. MERINOSELECT, which is a genetic evaluation service run by Meat & Livestock Australia based in Armidale NSW, analyses pedigree and performance recorded information on animals submitted by members to generate ASBVs and provides the best objective information available on Merino genetic trends. It is limited by the fact that only 30% of Australia's ram breeders are members although the percentage representation increases for large ram breeders and notably, 70% of Merino semen purchased in Australia is from MERINOSELECT members (See Table 9).

MERINOSELECT traits are grouped into fleece and carcass traits, visual traits, worm egg count, reproduction and indexes. The key traits are listed at the bottom of Table 1 and explanation of the traits and indexes can be found on the MERINOSELECT website http://www.sheepgenetics.org.au/Getting-started/ASBVs-and-Indexes.

MERINOSELECT commenced operations in 2006 when four separate databases agreed to combine to form a single national genetic evaluation service. The base year for the breech traits (breech wrinkle, breech cover and dag) is 2000, and the base year for all other traits is 1990.

Table 1 (Appendix 1) shows the MERINOSELECT average ASBVs since Year 2000. It includes the trends for the overall Merino breed as well as the three main Merino types, Superfine, Fine and Medium.

Key features of the ASBV genetic trends are:

Merino Breed:

- The total number of animals evaluated by MERINOSELECT each year continues to increase even though the total number of Merino ram flocks and rams sold has decreased since 2000
- The number of animals assessed for wrinkle and dag is increasing
- Body Weight is increasing
- Fat is steady but Muscle is increasing
- Fleece Weight is increasing
- Fibre Diameter is slightly increasing
- Worm Egg Count is decreasing
- Number of Lambs Weaned is steady (from an ASBV of -0.84% in 2000 to -0.55% in 2016)

- Breech Wrinkle is improving slowly (reducing), currently averaging -0.2
- Breech Wool Cover and Dag are steady.

Super Fine Type

- Fleece weight is increasing considerably while the increases for breech wrinkle and fibre diameter have been relatively restrained given the correlations they have with fleece weight. Worm egg count is improving. Current Breech Wrinkle ASBV is +0.1.

Fine Type

- Fleece weight is increasing while breech wrinkle has reduced (improved) by 0.2 score and the fibre diameter trend is steady. Worm egg count is improving. Current Breech Wrinkle ASBV is -0.2.

Medium type

- Fleece weight is increasing and fibre diameter has reduced (improved) while breech wrinkle has been held steady. Worm egg count is improving. Current Breech Wrinkle ASBV is -0.4.

These trends reflect the major economic pressures on woolgrowers over this period and the traits that ram buyers see as important when buying replacement flock rams. Fleece weight is increasing to fill more wool bales. Body weight is increasing as breeders chase higher growth rates. The losses due to worms are three times higher than the annual cost of flystrike (Lane et al, 2015) which is driving the improving worm egg count trends.

There are very mixed messages on the value of improving reproduction from a genetic perspective and it is a complex and costly trait to measure. The flat trends in Number of Lambs Weaned reflect these issues.

The numbers of dag and breech wrinkle records are increasing but genetic gains are modest for breech wrinkle and steady for dag. Breech wrinkle is improving slowly at the breed level and for the Fine wool Merino type. The macro trends in the three breech traits do not reveal what individual ram breeders have been and are doing, to breed naturally flystrike resistant Merinos.

There are a range of reasons for the breech trait genetic trends:

- It takes time for genetic momentum to develop and appear in the breed and type macro trends. ASBVs for breech wrinkle, breech cover and dag were released in late 2009, which is quite recent from a genetic perspective.
- New members joining MERINOSELECT and the increasing number of records are likely to be masking the gains being made by longer-term breech data collecting members (See Table 9).
- The FP+ and MP+ Indexes are promoting higher wrinkle animals (See Section 2).
- There is limited demand for lower wrinkle and lower dag animals as there is a lack of confidence that these types of Merinos can also be profitable. The current economic drivers are leading to gains in fleece weight, body weight and worm resistance.
- The above five issues have hidden the gains made by some individual ram breeders who for the last 5 to 10 years have been actively selecting for improved flystrike resistance and productivity.
- For some horned Merino breeders the demand for polled Merinos has added another trait to the selection criteria and slowed the gains in productivity and other welfare traits.
- There has been a focus on Yearling Fleece Weight rather than Adult Fleece Weight by ram breeders and this has misled breeders particularly with early maturing animals often associated with low wrinkle Merino types. They can, based on percentile ranges, have high early age fleece weight assessments and relatively lower adult fleece weight.
- A large number of Merino ram breeders are in low dag country where dags are only expressed infrequently (i.e. one in every 10 years or so), thus the number of dag records is less than for wrinkle. It is very difficult for these breeders to select for low dag.
- The future RD&E priorities are designed to further address these issues (See Section 4).

In previous RD&E Flystrike Updates (https://www.wool.com/sheep/welfare/breech-flystrike/latest-research/past-flystrike-prevention-rde-updates/) there were greater trends to lower wrinkle particularly in the Medium Wool Merino Type than now evident in the 21st April 2020 MERINOSELECT analysis. The change is also believed to be due to increasing animals in MERINOSELECT and more animals being recorded for the breech traits rather than a change in focus by these breeders.

The next section shows that there are leading sires emerging for high productivity and welfare, although more so in the medium Merino types than the others.

2. IMPACT OF INCREASING SELECTION PRESSURE FOR BREECH WRINKLE AND DAG ON CURRENT MERINOSELECT SIRES

Breech Wrinkle

The sire ASBVs required by a Merino breeder to breed progeny that are breech wrinkle score 2 or less, varies due to variations in the environment across Australia. In high wrinkle country target ASBVs are -0.8 to -1.0, in moderate wrinkle country -0.7 and low wrinkle country around -0.3. These are generalisations that need to be modified according to every woolgrower's own environment and their country's risk factors for breech strike. Across a range of environments and sheep types, trials have shown that mulesing reduces breech wrinkle by around 1.0 wrinkle score (range of from 1.5 to 0.5 scores with larger reductions on sheep with higher natural wrinkle) and dag by 0.4 dag score (Lloyd 2010 and Larsen 2012 R&D Technical Update, www.wool.com/flystrikelatest).

Table 3 was created by searching the MERINOSELECT database for all sires with more than 15 current progeny ranked in the Merino Production Plus Index order. There were 2,641 eligible sires in the open unrestricted search and the average ASBVs of the top 10 sires are listed in the table. Their average ASBV for ACFW is 34.5 and for breech wrinkle is +0.3. This shows that the average breech wrinkle ASBV of the 10 highest indexing MP+ sires is 0.5 score higher than the breed average (+0.3 compared to -0.2) which is discussed further below.

When the database search criteria on the upper limit of Breech Wrinkle was changed progressively from +0.2 to an improved -1.0, the average Adult Weight (AWT) ASBV in the top 10 sires moved from 5.4 to 9.2. Cross-checking this AWT range against the MERINOSELECT ASBV percentile bands in Table 2, a 5.6 AWT reflects rams in the top 30%, and a 9.1 AWT reflects rams in the top 5%. Adult Fleece Weight, however, fell but remained in the top 20% (+22), Fibre Diameter fell from the top 25% to the bottom 25% (-1.6 to -0.4), Breech Wrinkle improved from the bottom 15% to the top 1% (+0.3 to -1.1), Dag largely remained at breed average 50% (-0.1) and the MP+ Index fell from the top value but remained in the top 5% (224 to 177).

Table 3 All Merino Types, Average ASBVs of top 10 sires with more than 15 progeny based on MP+ Index for each search criteria

	Total No of Sires	Average ASBV of top 10 sires in each search						
Search Criteria	Meeting Criteria	AWT Kg	ACFW %	YFD	NLW	EBWR Sc	LDAG Sc	MP+ Index
Open Unresticted Search	2,641	5.4	34.5	-1.6	14.7	0.3	0.2	224
Breech Wrinkle<= +0.2	1,910	8.6	26.2	-1.2	17.4	-0.2	-0.1	215
Breech Wrinkle<= -0.1	1,362	9.7	24.5	-1.1	17.9	-0.3	0.0	215
Breech Wrinkle<= -0.4	809	10.4	23.4	-0.8	14.8	-0.6	-0.1	208
Breech Wrinkle<= -0.7	349	10.4	25.2	-0.2	10.4	-0.9	0.0	196
Breech Wrinkle<= -1.0	112	9.2	22.1	-0.4	8.5	-1.1	-0.1	177
Leading Low Wrinkle Sire A		11.6	34.3	-0.5	2.0	-1.3	0.0	186
Leading Low Wrinkle Sire B		10.2	28.4	-0.5	7.0	-1.1	-0.3	174
Leading Low Wrinkle Sire C		12.3	24.1	-1.0	20.0	-0.7	-	229
Leading Low Wrinkle Sire D		9.4	26.5	-0.4	11.0	-0.7	-0.2	202
Leading Low Wrinkle Sire E		14.3	26.2	0.3	19.0	-0.7	-0.2	214

Abbreviations; AWT - Adult Body Weight, ACFW - Adult Clean Fleece Weight, YFD - Yearling Fibre Diameter, NLW - Number of Lambs Weaned, EBWR Sc- Early Breech Wrinkle Score, LDAG Sc - Late Dag Score, MP+ - Merino Production Plus Index

Putting an upper limit of even +0.2 had a large effect in reducing the average wrinkle score of the 10 leading sires, from +0.3 to -0.2 with very little impact on the index. An upper limit of -0.1 reduced the wrinkle score from +0.3

to -0.3 again with very little impact on the index. Significant gains can be made by not considering just high wrinkle sires.

This database search demonstrates that breeding productive low breech wrinkle naturally breech strike resistant Merinos can be achieved in low dag country using existing sires based on the MP+ Index but with a large increase in Fibre Diameter and an increase in Adult Body Weight.

The ASBVs of some of the leading low breech wrinkle sires are shown in the bottom half of Table 3. Sire A is in the top 1% for fleece weight and top 1% for breech wrinkle. It is a young sire from a ram breeder in low dag country who is putting large selection pressure on high fleece weight and low breech wrinkle.

All the leading sires listed in Table 3 come from breeders with strong selection pressure on low breech wrinkle and high productivity with a focus on fleece weight. There are no leading sires by chance; they have been actively selected and bred by their owners. It should be noted that the fibre diameter for these sires is below average (ie broader) whilst the ASBVs for dag are average, suggesting that progress in breeding for breech strike resistance is likely to be easier for medium type Merinos in low dag environments.

Table 4 was created by searching all sires with more than 15 current progeny, ranked this time in the Dual Purpose Plus Index order. There were again 2,641 eligible sires in the open unrestricted search and the average ASBVS of the top 10 sires are listed. For the top 10 sires the average ASBV for Adult Clean Fleece Weight is lower at 29.6, Fibre Diameter higher at -1.2, yet the Breech Wrinkle ASBV is lower at -0.2 (breed average) compared to Table 3.

The upper limit search criteria for Breech Wrinkle was progressively changed from +0.2 to -1.0. The impact this had on the top 10 sires' average results based on the DP+ Index are listed in Table 4. In percentile terms Adult Weight remained in the top 1 to 5%, Fleece Weight fell from the top 1% to top 30%, Fibre Diameter fell from the top 40% to bottom 20%, Breech Wrinkle improved from average 50% to top 1-5%, Dag improved from the bottom 20% to top 30% and the DP+ Index remained constant from the near top value to the top 1%.

Table 4, All Merino Types, Average ASBVs of top 10 sires with more than 15 progeny based on DP+ Index for each search criteria

	Total No of Sires	Average ASBV of top 10 sires in each search						
Search Criteria	Meeting Criteria	AWT Kg	ACFW %	YFD	NLW	EBWR Sc	LDAG Sc	DP+ Index
Open Unresticted Search	2,641	9.1	29.6	-1.2	16.9	-0.2	0.1	234
Breech Wrinkle<= +0.2	1,910	9.9	22.8	-0.9	18.3	-0.4	0.0	232
Breech Wrinkle<=- 0.1	1,362	9.9	22.7	-0.9	18.3	-0.4	0.0	231
Breech Wrinkle<= -0.4	809	10.4	23.1	-0.8	15.5	-0.6	-0.1	226
Breech Wrinkle<= -0.7	349	10.2	21.1	-0.4	13.0	-0.9	-0.1	212
Breech Wrinkle<= -1.0	112	9.7	18.8	-0.3	10.2	-1.1	-0.2	196
Leading Low Wrinkle Sire A		11.6	34.3	-0.5	2.0	-1.3	0.0	193
Leading Low Wrinkle Sire F		11.9	27.3	0.5	5.0	-1.1	-0.1	210
Leading Low Wrinkle Sire G		9.4	26.5	-0.4	11.0	-0.7	-0.2	218
Abbreviations DP+ Index - Dual Purpose Plus Index								

Table 3 and 4 show that reducing breech wrinkle and increasing fleece weight is difficult if you are breeding fine wool sheep as there was a large corresponding increase in fibre diameter. There are very few ultrafine and superfine animals in MERINOSELECT with breech wrinkle scores less than zero so a similar process for Table 3 and 4 was not possible for Superfine Merino types.

So, for the Superfine Merino type, a different approach was undertaken. The upper fibre diameter limit was reduced progressively from -2.0 to -4.0. See Table 5. The Breech Wrinkle Score of the 10 highest FP+ indexing sires remained in the bottom 10% and very few sires were less than +0.3. High Indexing, low breech wrinkle superfine sires are difficult to find and breed, but ram breeders are taking up the challenge. There is evidence in the pedigree of the leading Superfine sires that 'outcross' sires are being used to reduce breech wrinkle, increase fleece weight, while attempting to minimise the increase in fibre diameter in Superfine Merinos.

Table 5. Ultra Fine/ Super Fine Type, Average ASBVs of top 10 sires with more than 15 progeny based on FP+ Index for each search criteria								
	Total No of Sires	Average ASBV of top 10 sires in each search						
Search Criteria	Meeting Criteria	AWT Kg	ACFW %	YFD	NLW	EBWR Sc	LDAG Sc	FP+ Index
Yearling FD <= -2.0	139	0.4	17.8	-3.0	-0.9	0.6	-0.1	179
Yearling FD <= -2.5	87	0.5	16.5	-3.1	-1.3	0.6	0.0	178
Yearling FD <= -3.0	37	1.2	11.0	-3.5	0.6	0.5	-0.1	172
Yearling FD <= -3.5	24	0.0	2.2	-4.0	-3.8	0.3	-0.1	158
Yearling FD <= -4.0	14	-0.7	-8.9	-5.2	-22.9	N/A	N/A	131
Leading Low Wrinkle Sire H		-1.5	-0.8	-3.6	n/a	-0.2	0.0	144
Leading Low Wrinkle Sire I		3.2	6.6	-4.3	1.0	-0.1	-0.2	160
Leading Low Wrinkle Sire J		0.7	10.6	-3.5	n/a	0.2	0.1	175
Abbreviations FP + Index - Fine Production Plus Index								

Dag

Putting selection criteria on current sires to reduce breech wrinkle had limited impact on reducing dags as seen in Tables 3, 4 and 5. This is expected as most of the leading sires are in low dag country, where dags are infrequently expressed. In high dag country dags can be the highest breech strike risk factor, particularly during winter and spring.

Table 6 was created using a similar search to Table 3, by searching all sires with more than 15 current progeny, ranked Merino Production Plus Index order but placing upper limits on dag. There were again 2,641 eligible sires in the open unrestricted search and the average ASBVS of the top 10 sires are listed. The dag score for these top 10 sires was +0.2 which is 0.3 dag score higher than the breed average (+0.2 compared to the breed average of -0.1).

The upper limit search criteria for Dag was progressively changed from -0.1 to -0.6. The impact this had on the top 10 sires average results based on the MP+ Index are listed in Table 6. In percentile terms Adult Weight remained in the top 10 to 20%, Fleece Weight fell from the top 1% to top 20%, Fibre Diameter fell from the top 40% to bottom 30%, Breech Wrinkle improved from the bottom 20% to top 40%, Dag improved from the bottom 10% to more than the top 1% and the MP+ Index fell from the near top value to top 10%.

This demonstrates that breeding productive low dag Merinos can be achieved using existing sires based on the MP+ Index, however growers need to be prepared for a fall in fleece weight and a large increase in fibre diameter. Again, it shows the difficulty of breeding low dag fine and super fine wool Merinos.

Table 6. All Merino Types, Average ASBVs of top 10 sires with more than 15 progeny based on MP+ Index for each search criteria								
	Total No of Sires	Average ASBV of top 10 sires in each search						
Search Criteria	Meeting Criteria	AWT Kg	ACFW %	YFD	NLW	EBWR Sc	LDAG Sc	MP+ Index
Open Unresticted Search	2,641	5.4	34.5	-1.6	14.7	0.3	0.2	224
Dags <-0.1	896	6.2	28.3	-1.7	16.3	0.0	-0.2	216
Dags <-0.2	472	7.2	26.3	-1.5	15.1	-0.1	-0.3	214
Dags <-0.3	229	6.9	31.8	-1.0	11.3	-0.2	-0.4	200
Dags <-0.4	100	5.8	25.6	-0.9	6.3	-0.1	-0.5	188
Dags <-0.5	42	7.0	15.7	-0.4	10.1	-0.4	-0.6	174
Dags <-0.6	24	7.7	15.4	-0.5	10.6	-0.4	-0.7	169
Leading Low Dag Sires		9.5	46.2	0.7	7.0	-0.2	-0.6	195
Leading Low Dag Sires		5.1	18.8	-2.3	0.0	-0.3	-0.5	187
Leading Low Dag Sires		6.0	25.5	0.1	5.0	-0.6	-0.5	180
Leading Low Dag Sires		8.4	7.6	-0.7	15.0	0.0	-0.9	172
Abbreviations; AWT - Adult Body Weight, ACFW - Adult Clean Fleece Weight, YFD - Yearling Fibre Diameter, NLW - Number of Lambs Weaned, EBWR Sc- Early Breech Wrinkle Score, LDAG Sc - Late Dag Score, MP+ - Merino Production Plus Index								

Breech Wrinkle, Dag and Fibre Diameter

Woolgrowers in high dag country need significant reductions in both breech wrinkle and dag to breed and create naturally resistant Merinos. Table 7 was created by searching all sires with more than 15 current progeny, ranked Merino Production Plus Index order with progressively lower upper limits on both breech wrinkle and dag. Fleece weight fell, fibre diameter rose while breech wrinkle and dag fell to naturally resistant levels for moderate wrinkle and dag country. Again, the rise in fibre diameter makes it difficult for woolgrowers who need fine wool with low diameter variation to breed for reduced breech strike.

A few sires could be found that were low in fibre diameter (less than -2.0) with moderate breech wrinkle and dag. However, of the total 2,641 eligible sires available on MERINOSELECT only one sire in the database is a trait leader (top 10%) for Wrinkle, Dag, Adult Fleece Weight and the MP+ Index yet its Fibre Diameter is high at +0.5.

Table 7. All Merino Types, Average ASBVs of top 10 sires with more than 15 progeny based on MP+ Index for each search criteria

	Total No of Sires	Average ASBV of top 10 sires in each search						
Search Criteria	Meeting Criteria	AWT Kg	ACFW %	YFD	NLW	EBWR Sc	LDAG Sc	MP+ Index
Breech Wrinkle <-0.2 and Dags<-0.1	606	9.5	22.7	-0.9	16.3	-0.4	-0.3	209
Breech Wrinkle <-0.4 and Dags<-0.2	268	10.7	18.6	-0.9	14.4	-0.7	-0.3	202
Breech Wrinkle <-0.5 and Dags<-0.3	103	9.7	20.6	-0.9	8.7	-0.7	-0.4	188
Breech Wrinkle <-0.6 and Dags<-0.4	39	8.6	12.6	-0.5	7.7	-0.8	-0.5	165
Breech Wrinkle <-0.2, Dags<-0.1 and YFD<-2	30	4.3	18.1	-2.3	2.0	-0.3	-0.2	175
Breech Wrinkle <-0.4, Dags<-0.2 and YFD<-2	9	6.6	5.9	-2.4	1.6	-0.6	-0.4	155
Trait Leader Wrinkle, Dag, Fleece Wt & MP+	1	11.6	37.5	0.5	8.0	-1.0	-0.3	181

Breeding naturally breech strike resistant high indexing fine wool Merinos will take some considerable time. Fine wool breeders that already manage their flystrike risk without mulesing have long used other management strategies to help reduce the risk of breech strike. Approaches include regular long acting chemical treatments, additional crutching and shearing and closer supervision of their animals. Much of the fine wool environment is low phenotypic wrinkle country; cold, wet, windy and wormy where Breech Wrinkle ASBV's of -0.3 to -0.5 are likely to be required. Importantly from the Breeding for Breech Strike Resistance project, every 0.1 score reduction in any trait, wrinkle, cover, stain and or dags reduces the risk of breech strike, irrespective of the starting natural score.

3. POTENTIAL OUTCOMES OF THE MERINO LIFETIME PRODUCTIVITY PROJECT FOR BREEDING NATURALLY FLYSTRIKE RESISTANT MERINOS

The MLP project is a $12M, 10-year partnership between AWI, the Australian Merino Sire Evaluation Association (AMSEA), 5 Breeder Site Committees, 5 Site Hosts and nominating Stud Merino breeders. AWI is providing $7M and the remaining $5M comes from Site Hosts, Site Committees and Entrants.

The Site Hosts and Committees are:

Balmoral – "Tuloona", Harrow, Vic (Tuloona Pastoral and Balmoral Breeders Site Committee)
Pingelly - UWA Farm Ridgefield, Pingelly WA (Murdoch University and Federation of Performance Sheep Breeders (WA Branch))
MerinoLink - "The Vale", Temora, NSW (MerinoLink Limited, Moses and Son, Bluechip Livestock)
Macquarie - Trangie Agricultural Research Centre, Trangie, NSW (NSW DPI and Macquarie Sire Evaluation Association)
New England - "Chiswick", Uralla, NSW (CSIRO and New England Merino Sire Evaluation Association)

The MLP project (2015-2025) is running at the above sites where sire evaluation trials are conducted for the first two years and then continues to track the performance of the 5,700 F1 ewe progeny from 134 sires as they proceed through four to five joinings, lambings and annual shearings. A full suite of assessments are being taken including visual trait scoring, objective assessment of a broad range of traits, classer gradings and index evaluations. 134 sires were artificially inseminated to 90 ewes each. They were carefully selected from hundreds of industry nominations to allow a comprehensive examination of all the factors that might influence lifetime productivity, and to generate results that will be both industry representative and relevant.

Sire selection was targeted to minimise bias. The target was to achieve a balance across each site and drop, along with an overall strategy that the project's sire list across all sites would meet the following criteria:

- **Industry representative**: a balance between horn poll rams, different skin/wool types, rams with and without Australian Sheep Breeding Values (ASBVs).
- **Impact rams:** Significant show performance results or widely used AI sires in industry (with or the without ASBVs).
- **Genetically representative**: Selected from four main genetic performance groups within the MERINOSELECT database. These groups were identified using a unique analysis of the MERINOSELECT database that included progeny of sires used between 2006 and 2016. The number of animals from each group aimed to match the proportion of each group in MERINOSELECT.
- **Performance range**: High and low performance for key traits based on ASBV percentile band tables.
- **Fleece value**: Sires predicted to maintain, increase or decrease fleece value over time.
- **Sire Age**: A balance of young (progeny yet to be evaluated) and older rams.

The final sire lists at each site were generated in consultation with each Sire Evaluation Site Committee plus the MLP Industry Steering Committee. (There is greater detail at https://merinosuperiorsires.com.au/wp-content/uploads/2020/05/MLP-Sire-Selection-Process-and-Stocktake-FINAL.pdf).

This unique and extensive dataset will be used to enhance existing Merino breeding and selection strategies, for both ram sellers and buyers, to deliver greater lifetime productivity and woolgrower returns.

All sires except for three, currently have progeny assessed as pre lambing 2-year old's and over the next 4 years all the adult production records for all traits will be recorded for all sires. By August 2020, all sires will have progeny results collected at 2 years old. The formal project assessment will be between the sires using within project Flock Breeding Values (FBVs) with all the repeat adult data which will not occur until 2025.

All the MLP data is available for use in MERINOSELECT and the repeat MLP adult data will be an important asset for ongoing RD&E in Merino Genetic Benchmarking. Data from the MLP Add-On projects, particularly regarding Resilience (CSIRO) and Economic Evaluation of Genetic Gain (Murdoch University) will add to the data flow and cooperation between the MLP projects and MERINOSELECT (See Section 4).

Below is the current (April 2020) ASBV data on the 134 MLP sires which combines both the MLP data with across MERINOSELECT members on farm data. Tables 8 to 12 show that the balance in sire performance has been achieved and is truly Industry representative. Table 8 shows that there is a wide variation in the ASBVs of the 134 MLP sires particularly in the welfare traits of fat, worm egg count, breech wrinkle and dag.

Table 8. Range in performance of MLP sires based on current ASBVs (April 2020)

	ycfw	**acfw**	**yfd**	**yss**	**Ysl**	**ywt**	**awt**	**yfat**	**yemd**	**ywec**	**ebwr**	**edag**	**nlw**	**DP+**	**MP+**	**FP+**
Min	-23	-30	-4.3	-6.4	-17.3	-3.8	-4.4	-2.9	-3.0	-78	-1.6	-0.5	-24%	88	96	93
Max	43	43	2.2	11.1	26.4	12.9	12.1	2.9	3.9	141	1.4	0.7	23%	230	226	195
Ave	**21**	**19**	**-1.3**	**0.7**	**5.5**	**5.8**	**4.5**	**-0.2**	**0.1**	**9**	**0.0**	**0.0**	**-1%**	**157**	**159**	**148**
MS#	*15*	*13*	*-1.0*	*0.6*	*7.1*	*4.8*	*3.9*	*0.1*	*0.3*	*-16*	*-0.2*	*0.0*	*1%*	*144*	*143*	*136*

the average of the 2018 drop Merino progeny in MERINOSELECT

Table 9 compares the current differences between the sires based on MERINOSELECT membership status. It shows there are differences between the two in some traits, but also reasonable similarity in others. The indexes are well aligned now, with the ongoing caveat that there are still four years to run in the MLP project and the best comparison will be at the end of the project using MLP only data. Not surprisingly, visual selection for key traits that are "visually expressed", highly heritable and are not antagonistic (don't work against each other) combined with the aid of raw data has been very successful as Table 9 indicates for fleece weight, body weight and fibre diameter. Where the number of traits under selection increase, where there are a number of antagonist traits under selection and/or there are a number of "non visual traits", using ASBV's appears to be advantageous i.e. for fat, muscle, worm egg count, wrinkle and reproduction traits. (Note Table 9 only shows a comparison of MERINOSELECT membership status, members within each status can also have very differing selection strategies).

Table 9. Number of sires entered based on MERINOSELECT membership status (April 2020)

Status	No	ycfw	acfw	yfd	yss	ysl	ywt	Yfat	yemd	ywec	ebwr	edag	nlw	DP+	MP+	FP+
Non	35	22	23	-1.1	1.2	0.6	5.2	-0.7	-0.5	32	0.2	0.05	-3%	152	158	146
Member*	99	21	17	-1.3	0.6	7.3	6.0	0.0	0.3	2	0.0	-0.01	0%	159	160	149
Total	**134**	**21**	**19**	**-1.3**	**0.7**	**5.5**	**5.8**	**-0.2**	**0.1**	**9**	**0.0**	**0.00**	**-1%**	**157**	**159**	**148**

*Includes one Dohne

Table 10 shows the broad range of Breech Wrinkle of the MLP sires. Seventeen sires average -1.1 for Breech Wrinkle and eighteen sires average +1.0. The occurrence of the different breech wrinkle scores along with other traits can be seen and replicates outcomes above in Section 2 when upper limits were placed on Breech Wrinkle on the current 2,631 sires in MERINOSELECT. There are large differences in fibre diameter, fat, muscle and NLW between the breech wrinkle categories.

Table 10. Average ASBV Performance of Sires Based on Breech Wrinkle (EBWR) ASBV (April 2020)

EBWR ASBV	Count	ycfw	acfw	yfd	Yss	ysl	ywt	awt	yfat	yemd	ywec	ebwr	edag	nlw
< -0.7	17	19	12	-0.1	0.5	18.0	7.9	6.6	1.0	1.6	-7	-1.1	-0.07	5%
-0.6 to -0.1	43	20	17	-1.2	1.1	7.4	7.2	6.0	0.2	0.6	-1	-0.3	-0.06	2%
0 to +0.3	28	27	24	-1.2	1.0	6.1	6.3	5.0	-0.5	-0.2	25	0.1	0.01	0%
+0.4 to +0.7	28	22	21	-1.8	1.0	-0.1	4.3	3.0	-0.6	-0.7	14	0.5	0.01	-2%
>0.8	18	16	18	-2.2	-0.7	-2.9	2.0	0.5	-1.0	-0.8	19	1.0	0.22	-10%
Total	**134**	**21**	**19**	**-1.3**	**0.7**	**5.5**	**5.8**	**4.5**	**-0.2**	**0.1**	**9**	**0.0**	**0.00**	**-1%**

Table 11 shows a good range in Dag for the MLP sires. Twelve sires average -0.4 for and fifteen sires average +0.4 and the occurrence of the different dag scores with the other traits.

Table 11. Average ASBV Performance of Sires Based on Dag ASBV (April 2020)

EDAG ASBV	Count	ycfw	acfw	yfd	yss	ysl	ywt	awt	yfat	yemd	ywec	Ebwr	edag	nlw
< -0.4	12	20	14	-0.9	1.2	7.8	7.8	6.3	0.7	0.8	-22	-0.2	-0.4	5%
-0.2 to -0.1	46	20	17	-1.5	0.6	6.3	5.8	4.6	0.0	0.4	9	-0.2	-0.1	2%
0	23	23	21	-1.0	0.2	6.6	5.5	4.3	-0.3	-0.1	6	0.0	0.0	-3%
+0.1 to +0.2	38	23	20	-1.2	1.1	5.3	5.9	4.5	-0.4	-0.3	21	0.2	0.1	-1%
>0.3	15	20	21	-1.8	0.7	0.2	4.4	3.1	-0.6	-0.4	13	0.6	0.4	-6%
Total	**134**	**21**	**19**	**-1.3**	**0.7**	**5.5**	**5.8**	**4.5**	**-0.2**	**0.1**	**9**	**0.0**	**0.0**	**-1%**

Table 12 shows the broad range of Fibre Diameter associated with the sires in the MLP project. The occurrence of low Fibre Diameter with high Breech Wrinkle is again illustrated. Twelve sires average -3.2 for fibre diameter and eight sires average +0.8. The impact of the different diameter groups impacts fleece weight at the low end only, but across the board for weight, fat, muscle and wrinkle.

Table 12. Average ASBV Performance of Sires Based on Fibre Diameter (FD) ASBV (April 2020)

FD ASBV	Count	ycfw	acfw	yfd	yss	Ysl	ywt	awt	yfat	yemd	ywec	ebwr	edag	nlw
SF <-2.8	12	8	5	-3.2	-1.4	-4.0	2.6	1.2	-0.4	-0.2	-11	0.8	0.08	-8%
F -2.7 to -1.8	33	20	19	-2.2	-0.5	2.3	4.7	3.2	-0.3	-0.1	15	0.2	-0.03	0%
FM -1.7 to -0.8	47	23	21	-1.2	0.6	5.2	5.9	4.6	-0.5	-0.3	22	0.0	0.05	-1%
M -0.7 to +0.3	34	24	20	-0.3	2.2	10.1	7.4	6.2	0.2	0.6	-2	-0.2	-0.04	1%
S > +0.4	8	26	20	0.8	3.2	15.5	7.9	6.9	0.7	1.4	3	-0.7	-0.04	1%
Total	**134**	**21**	**19**	**-1.3**	**0.7**	**5.5**	**5.8**	**4.5**	**-0.2**	**0.1**	**9**	**0.0**	0.00	**-1%**

The variability in traits of the MLP sires is large and when grouped on breech wrinkle, dag and fibre diameter they mirror the impact of changing the upper limits in the animals search functions on the 2,641 current sires in Section 2.

Of specific interest regarding breeding naturally flystrike resistant Merinos, will be the lifetime performance of high and low sires for breech wrinkle, dag, breech cover, fleece weight, reproduction, fat and muscle along with the comparison of the accuracy of early in life productivity indicators of productivity versus actual lifetime performance. Woolgrowers will be able to witness the relative profitability of naturally flystrike resistance sheep and the project will answer questions and concerns that ram breeders and buyers have about the consequences of selecting for flystrike and welfare traits (i.e. survival, polls, worm egg count and the breech traits).

4. FUTURE RD&E PRIORITIES TO ASSIST WOOLGROWERS BREED NATURALLY FLYSTRIKE RESISTANT MERINOS

The Breeding for Breech Strike Resistance Flocks ran from 2005 to 2015. Since 2008 they have been a major contributor to the biennial Breech Strike Technical RD&E updates and to the commercial release of ASBVs for Breech Wrinkle, Dag and Breech Cover in late 2009. This was followed by two phases of a project investigating associations between 1,526 animals high density DNA genotypes and their flystrike records and another project investigating the role that odour and skin bacteria might play in attracting gravid flies to lay their eggs on some and not other sheep. The genotype study could not find any single genes of major effect, but that a genome wide analysis was likely to have benefits although further R&D is required.

The odour study could not find the specific odours that attracted the gravid fly although it was shown the sniffer dogs could determine a difference between wool samples taken from resistant and susceptible sheep some 18 months after the flystrike trial period. These outcomes have been reported at the Technical Updates from 2008 to 2018.

AWI does have two current projects looking at improving resilience in the Merino and improving productivity and profitability measures in Merinos. Both are well aligned with breeding naturally flystrike resistant, highly productive Merinos. The Improving Resilience in Merino's project is with the CSIRO Armidale and the Genetic Evaluation of Productivity Efficiency and Profit project with Murdoch University.

The Breeding and Selection pillar of the Flystrike Strategy has entered a phase with an increased focus on development and extension. Priorities for this work include:

- Further development to allow the use of neck wrinkle to create Breech Wrinkle ASBVs for very low breech wrinkle Merinos and Dohnes. (Body wrinkle already contributes to breech wrinkle).
- Investigations into other flystrike resistance related indicator traits
  The breech flystrike causal web captures a number of risk factors for flystrike that could be captured by ASBVs to enable growers to select for them, potentially reducing their overall flystrike risk. Work to understand the genetic heritability, and therefore appropriateness of developing ASBVs for risk factors such as fleece rot, urine stain and faecal consistency will be investigated
- Development of a Survival / Longevity ASBV

Survival and longevity are complicated traits but are important welfare and economic considerations. Data from the MLP project will be needed to create these new traits.

- Creation of Flystrike Resistance and Improved Welfare influenced Indexes
  The MP+ and FP+ Indexes are not influenced by breech wrinkle or dag and as a result these indexes are tending to inadvertently promote higher wrinkle animals due to their association with increased fleece weight. GRASS Merino's have developed an index that puts pressure on reducing breech wrinkle and this will be released for public use in 2020. Development of further welfare influenced Indexes is planned.
- Improved estimation of adult age traits from young age assessments
  Current genetic advice is for ram breeders to collect 1) pedigree, 2) yearling fleece and carcass assessments 3) late hogget fleece and carcass assessments and 4) repeat adult reproduction records, although this recommendation may change with outcomes of the MLP project. This is time consuming and expensive and most ram breeders do not collect hogget or adult records, However, in the meantime, better predictions of lifetime performance using yearling data could assist with optimising profitability and welfare gains for early and late maturing animals
- Cost benefit assessment of a virtual genomic flock for flystrike / welfare traits
  Woolgrowers actively protect animals from flystrike so direct assessment of this trait is difficult and complex. AWI is investigating the option of a virtual genomic flock such that when a breeder does get a struck animal its genotype and that from a non-struck animal in the same mob could be used to develop a predictive Flystrike ASBV. The same approach may also assist with Survival and Longevity
- Improving sampling protocols for Worm Egg Count ASBV
  As ram breeders have made genetic gains it has become harder to meet the minimum level of worms required to obtain a good assessment. The level required can also impact on the growth rate and condition of the animals. A new method, the mini-FLOTAC, of assessing worm egg counts, which presents a superior sensitivity compared to the current method, is being trialed

FURTHER INFORMATION

Further information on AWI's Sheep Genetics projects is available on the AWI website at https://www.wool.com/sheep/genetics/.

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For 5 years Geoff was Manager of the Trangie Agricultural Research Centre and ran the Fleece Management Lab for 2 years. Geoff held the position of Livestock Operations Manager for the Twynam Agricultural Group for 11 years before joining Australian Wool Innovation in 2007 where he has been Program Manager in Genetics, Breech Strike and Sheep Health and Welfare.

APPENDIX 1.

Table 1 MERINOSELECT ASBVs Trends by Overall Breed and Merino Type since 2000

Merino Breed

Year of	Animals	ywt	awt	yfat	yemd	ygfw	agfw	acfw	yfd	yss	ywec	nlw	ebwr	ebwr	ebcov	ldag	ldag	FPP	MPP	DPP
Birth	Recorded	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	Records	ASBV	ASBV	Records	ASBV	ASBV	ASBV
drop	head	kg	kg	mm	mm	%	%	%	micron	N/Kt	%	%	score	head	score	score	head	index	index	index
2000	44,254	0.0	-0.5	0.0	0.1	0.9	0.0	2.7	-1.2	-0.5	4.6	-0.84	0.0		-0.1	0.0	436	118	117	115
2002	78,177	0.5	0.1	0.0	0.2	0.3	-0.2	2.4	-1.3	-0.4	0.6	-1.60	-0.1		0.0	0.0	768	121	118	117
2004	73,653	0.9	0.5	0.1	0.3	0.6	-0.2	2.7	-1.3	-0.2	-1.7	-1.75	-0.1		0.0	0.0	640	121	119	119
2006	62,734	1.6	1.2	0.1	0.3	2.5	0.9	4.0	-1.2	0.2	-6.7	-2.23	-0.1	2,654	0.0	0.0	2,728	123	123	122
2008	67,757	1.9	1.4	0.1	0.3	4.1	1.9	5.0	-1.2	0.3	-6.0	-1.56	-0.1	13,691	0.0	0.0	7,139	126	126	125
2010	69,743	2.6	2.0	0.1	0.4	5.2	2.8	6.3	-1.1	0.3	-8.1	-1.73	-0.1	19,291	-0.1	0.0	5,277	127	128	128
2012	99,453	3.0	2.4	0.1	0.4	5.7	3.3	7.1	-1.1	0.4	-9.8	-1.24	-0.1	26,636	-0.1	0.0	8,680	128	130	130
2014	116,934	3.3	2.6	0.1	0.3	7.6	4.7	8.8	-1.1	0.5	-11.7	-0.63	-0.1	20,501	-0.1	0.0	11,773	130	134	133
2016	118,174	4.0	3.2	0.1	0.4	9.5	6.1	10.6	-1.0	0.6	-14.3	-0.55	-0.2	23,229	-0.1	0.0	10,682	132	137	138
2018	127,787	4.7	3.9	0.1	0.4	11.8	7.8	12.7	-1.0	0.5	-17.2	0.76	-0.2	27,787	-0.1	-0.1	7,982	136	143	145

Super Fine Wool Merino Type

Year of	Animals	ywt	awt	yfat	yemd	ygfw	agfw	acfw	yfd	yss	ywec	nlw	ebwr	ebwr	ebcov	ldag	ldag	FPP	MPP	DPP
Birth	Recorded	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	Records	ASBV	ASBV	Records	ASBV	ASBV	ASBV
2000	13,541	-2.0	-2.4	0.2	0.4	-10.0	-10.4	-8.9	-1.9	-0.4	3.7	-0.90	0.0		0.0	0.1	292	113	102	99
2002	18,457	-1.9	-2.2	0.1	0.3	-10.3	-10.5	-9.4	-2.0	-0.5	-1.2	-2.42	0.0		0.0	0.0		115	103	99
2004	21,364	-1.9	-2.1	0.2	0.4	-10.2	-10.2	-8.8	-2.0	-0.5	-2.1	-3.07	0.0		0.0	0.0	195	116	104	100
2008	17,642	-0.6	-1.1	0.1	0.3	-4.9	-6.4	-4.6	-2.0	0.0	-3.2	-4.59	0.1	2,236	0.1	0.0	1,347	123	115	109
2010	14,200	-0.3	-0.9	-0.1	0.2	-3.3	-4.8	-2.6	-2.0	-0.2	-15.5	-4.98	0.1	1,452	0.1	0.0	1,005	126	117	111
2012	20,319	0.0	-0.5	-0.1	0.1	-3.6	-5.0	-2.5	-2.0	0.0	-7.2	-5.17	0.1	2,893	0.1	0.0	1,742	127	118	113
2014	17,571	0.6	-0.1	-0.1	0.2	0.1	-1.9	0.6	-1.9	0.3	-13.9	-3.08	0.1	2,366	0.0	0.0	1,419	130	124	119
2016	16,085	1.7	1.0	-0.1	0.2	2.7	0.0	2.9	-1.8	0.2	-17.2	-3.71	0.1	2,431	0.1	0.0	2,269	132	129	125
2018	13,199	2.9	1.9	0.0	0.4	6.6	3.0	6.7	-1.7	0.3	-16.6	-4.84	0.1	1,561	0.0	0.0	1,384	137	137	135

Fine Wool Merino Type

Year of	Animals	ywt	awt	yfat	yemd	ygfw	agfw	acfw	yfd	yss	ywec	nlw	ebwr	ebwr	ebcov	ldag	ldag	FPP	MPP	DPP
Birth	Recorded	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	Records	ASBV	ASBV	Records	ASBV	ASBV	ASBV
2000	30,306	0.5	0.0	-0.2	-0.1	5.2	4.3	7.4	-1.1	-0.9	6.6	-0.11	0.0		0.0	0.0	144	122	124	123
2002	53,280	0.8	0.5	0.0	0.2	3.2	3.0	5.9	-1.2	-0.5	0.2	-1.93	0.0		0.0	0.0	768	124	124	123
2004	47,671	1.3	0.9	-0.1	0.1	4.2	3.9	7.0	-1.2	-0.2	-3.3	-2.51	0.0		-0.1	0.0	445	125	126	125
2006	37,384	1.9	1.5	0.0	0.2	6.1	4.5	8.1	-1.0	0.2	-10.3	-2.38	-0.1	1,117	-0.1	-0.1	1,979	126	128	127
2008	40,024	2.3	1.7	0.0	0.2	7.4	5.0	8.6	-1.1	0.3	-8.2	-0.82	-0.1	9,361	-0.1	0.0	4,539	129	132	131
2010	40,450	2.8	2.2	0.1	0.4	7.2	5.0	8.8	-1.1	0.5	-4.3	-0.74	-0.1	15,163	-0.1	0.0	3,777	130	133	133
2012	58,737	3.1	2.5	0.0	0.3	7.5	5.3	9.3	-1.1	0.4	-9.2	-0.75	-0.1	20,806	-0.1	0.0	6,250	131	134	134
2014	64,250	3.3	2.7	0.0	0.3	9.0	6.1	10.5	-1.1	0.6	-10.3	-0.41	-0.1	16,845	-0.1	0.0	7,772	133	137	136
2016	61,807	4.3	3.5	0.1	0.4	11.0	7.2	11.9	-1.0	0.8	-15.3	0.38	-0.2	16,676	-0.2	-0.1	6,520	136	142	142
2018	66,612	4.7	3.9	0.1	0.4	13.1	8.7	13.7	-1.1	0.7	-17.3	2.87	-0.2	14,752	-0.1	-0.1	3,906	140	147	148

Medium Wool Merino Type

Year of	Animals	ywt	awt	yfat	yemd	ygfw	agfw	acfw	yfd	yss	ywec	nlw	ebwr	ebwr	ebcov	ldag	ldag	FPP	MPP	DPP
Birth	Recorded	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	Records	ASBV	ASBV	Records	ASBV	ASBV	ASBV
2000	4,926	2.1	1.8	0.0	0.0	6.8	5.2	8.9	-0.3	0.8	2.6	0.74	-0.4		-0.1	0.0		115	120	122
2002	10,757	2.3	1.9	0.0	0.1	6.8	4.7	8.7	-0.6	0.0	8.6	-0.09	-0.3		0.0	0.0		118	123	124
2004	9,857	3.5	3.2	0.2	0.5	7.1	4.4	8.6	-0.4	0.3	9.7	0.99	-0.3		-0.1	0.0		118	125	130
2006	9,067	4.0	3.7	0.2	0.5	7.2	4.4	8.3	-0.5	0.3	-13.0	1.91	-0.3	1,558	-0.1	0.1		119	127	131
2008	9,039	4.1	3.7	0.3	0.6	7.1	4.2	8.3	-0.5	0.5	-3.7	1.02	-0.3	2,244	-0.1	0.0	863	120	127	132
2010	13,865	4.8	4.0	0.3	0.6	8.0	4.6	8.8	-0.6	0.0	-9.8	0.16	-0.3	3,112	-0.1	0.0	482	122	129	133
2012	16,036	4.8	4.1	0.3	0.6	8.5	5.0	9.2	-0.7	0.7	-15.5	1.76	-0.4	4,726	-0.1	0.0	688	126	133	137
2014	25,485	4.5	3.7	0.2	0.4	9.4	6.2	10.8	-0.8	0.5	-18.0	1.40	-0.4	1,909	-0.2	-0.1	271	129	136	138
2016	26,216	4.8	3.9	0.1	0.4	10.6	7.5	12.5	-0.8	0.4	-22.5	-0.02	-0.4	504	-0.1	0.0		129	138	140
2018	29,260	5.7	4.8	0.2	0.4	13.0	9.4	15.2	-0.7	0.3	-18.7	0.63	-0.4	3,366	-0.1	0.0	234	133	144	147

Abbreviations; ywt Yearling Body Weight; awt Adult Body Weight; yfat Yearling Fat; yemd Yearling Eye Muscle Depth; ygfw Yearling Greasy Fleece Weight; agfw Adult Greasy Fleece Weight; acfw Adult Clean Fleece Weight; yfd Yearling Fibre Diameter; yss Yearling Staple Strength; ywec Yearling Worm Egg Count; nlw Number of Lambs Weaned; ebwr Early Breech Wrinkle; ebcov Early Breech Cover; ldag Late Dag; FPP Fibre Production Plus Index; MMP Merino Production Plus Index; DPP Dual Purpose Plus Index.

Scource MERINOSELECT

Table 2 MERINOSELECT ASBV Percentiles (21st April 2020)

Percentile	ywt	awt	ycf	yemd	ygfw	agfw	acfw	yfd	yss	ywec	nlw	ebwr	ebcov	ldag	FPP	MPP	DPP
Band	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV	ASBV
%	kg	kg	mm	mm	%	%	%	micron	N/Kt	%	%	score	score	score	index	index	index
Top Value	19.9	21.9	7.1	5.0	49.5	39.7	51.1	-6.1	14.7	-95	25	-1.7	-1.5	-0.8	201	224	243
Top 1%	11.9	11.4	2.0	3.0	30.2	25.1	33.1	-3.3	7.0	-83	15	-1.2	-0.8	-0.4	172	188	194
Top 5%	9.8	9.1	1.4	2.3	24.8	20.4	27.8	-2.5	5.0	-63	10	-0.9	-0.6	-0.3	161	174	178
Top 10%	8.7	8.0	1.1	1.9	22.0	18.0	24.8	-2.2	3.9	-52	7	-0.8	-0.4	-0.3	155	166	169
Top 20%	7.3	6.6	0.8	1.4	18.5	14.8	21.0	-1.8	2.7	-40	5	-0.6	-0.3	-0.2	148	158	159
Top 30%	6.4	5.6	0.5	1.0	15.9	12.4	18.2	-1.5	1.9	-32	3	-0.5	-0.2	-0.2	143	152	153
Top 40%	5.5	4.7	0.3	0.6	13.6	10.3	15.7	-1.2	1.2	-24	2	-0.3	-0.2	-0.1	139	147	148
Top 50%	4.8	3.9	0.1	0.4	11.4	8.3	13.2	-1.0	0.6	-16	1	-0.2	-0.1	-0.1	135	143	143
Top 60%	4.0	3.1	-0.1	0.1	9.2	6.2	10.8	-0.8	-0.1	-8	0	-0.1	0.0	0.0	132	138	139
Top 70%	3.1	2.3	-0.3	-0.2	6.8	4.0	8.2	-0.6	-0.8	-1	-2	0.0	0.0	0.0	128	133	134
Top 80%	2.1	1.2	-0.5	-0.5	4.0	1.4	5.1	-0.3	-1.6	10	-3	0.2	0.1	0.1	123	127	129
Top 90%	0.7	-0.2	-0.8	-0.9	-0.1	-2.2	0.8	0.2	-2.8	28	-5	0.4	0.2	0.2	115	119	121

Scource MERINOSELECT

GAP EVALUATION OF PAIN ALLEVIATION RESEARCH

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SUMMARY
Increasing societal and customer demands to provide animals with 'a life worth living' continue to apply pressure on industry to alleviate pain associated with husbandry practices, injury and illness. Over the past 15-20 years, there has been considerable research effort, with funding in the order of AU$9 million from AWI, to better understand and develop mitigation strategies for painful husbandry procedures in sheep. This stocktake review of research literature published between 2000 and 2019 was undertaken so that AWI can strategically focus future project activities on the most important challenges, and the avenues which offer the greatest potential to be incorporated into industry best practice in pursuit of continuous improvement.

PROJECT REPORT
Social values, across a wide spectrum of concerns, continually change. For animals, the widespread view that animals deserve 'a life worth living' applies pressure on industry to improve pain management for husbandry practices, injury and illness. Notably, over the past 15-20 years, there has been considerable research effort across research organisations, industry (including funding in the order of AU$9 million from AWI) and commercial pharmaceutical companies, to better understand the impact of, and to develop mitigation strategies for painful husbandry procedures in sheep. This international research effort, and particularly the recent collaborative approach of industry and commercial companies, has led to the successful launch in Australia of two registered Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) – Ilium® Buccalgesic OTM® and Metacam 20®, and two local anaesthetic products for sheep – Tri-Solfen® and NUMNUTS®. However, even with multimodal approaches to analgesia using both Local Anaesthetic and NSAID, pain is not obliterated, and the challenge of pain mitigation and phasing out of painful husbandry practices remains. To ensure the sheep industry stays abreast of opportunities for improving pain control, it is important to take stock periodically of progress in international research into pain relief in other species so that AWI can strategically focus on the most important challenges, and identify avenues which offer the greatest potential to be incorporated into industry practice in a process of continuous improvement.

The project undertook a stocktake of published research into the welfare impacts of castration, tail docking and mulesing; alternatives to these procedures; and potential pain relief strategies. The stocktake catalogue included research on:

1. Mulesing and its alternatives; castration and tail docking (ring and knife, hot/cold); Laparoscopic Artificial Insemination and shearing cuts. Relevant literature pertaining to other husbandry procedures were considered.
2. Pain mechanisms and pain mitigation strategies, considering the context of the procedure and the pain mechanisms triggered. 'Pain mitigation strategies' examined both pharmaceutical agents and delivery mechanisms.
3. Methods of assessing pain and analgesic efficacy.

This was quite a broad remit – when the term 'analgesia' is entered into one of the literature database search engines (in this case Web of Science®, Thomson Reuters), 73,045 potential articles are returned (Figure 1). This enormous number of research articles is evidence that the issue of pain relief has not yet been solved, for any species. In order to bring the task into a more manageable size, it was decided to focus only on articles published from 2000 to 2019, there having been some very comprehensive reviews published in the early 2000's. Merely reducing the time-frame in the literature search dropped the number of articles to 52,302 (Figure 2).

Furthermore, it is important to focus on the important underlying questions to the review We also didn't really need to cover other review articles, other than recording their existence for cross-referencing purposes, or to set a context, and we didn't need to cover 'opinion' or 'discussion' articles that have no experimental content. There are also language aspects: many languages are beyond the skills of the immediate research team, so a sensible criterion was to exclude 'language other than English'. This led to the development of strict inclusion-exclusion criteria (Table 1), against which each title and abstract was assessed as an initial step.

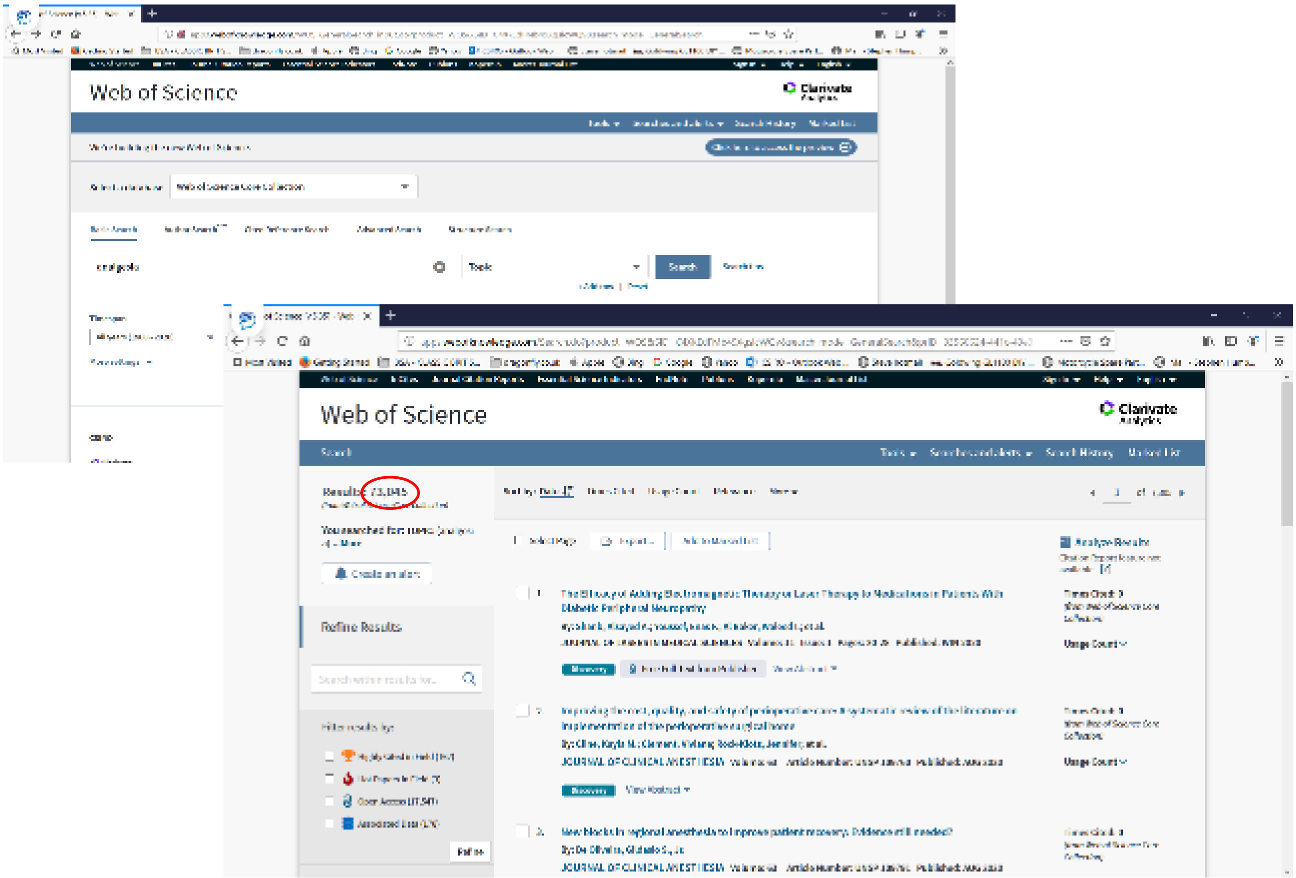

Figure 1. A simple search on Web of Science®, using the search term 'analgesia' returned over 73,000 potential articles.

Table 1. Literature Search Criteria.

Databases to search	Web of Science core collection PubMed MedLine Scopus
Inclusions	Research Papers Analgesic agents Husbandry procedures Livestock Companion animals Humans
Exclusions	Policy documents Reviews (except as a means to identify other research) Philosophical/opinion papers Patents General anaesthesia Papers published prior to 2000* Language other than English
*Although the focus of the review is on papers published between 2000 and 2019, some earlier papers have been cited in this report in order to provide context.	

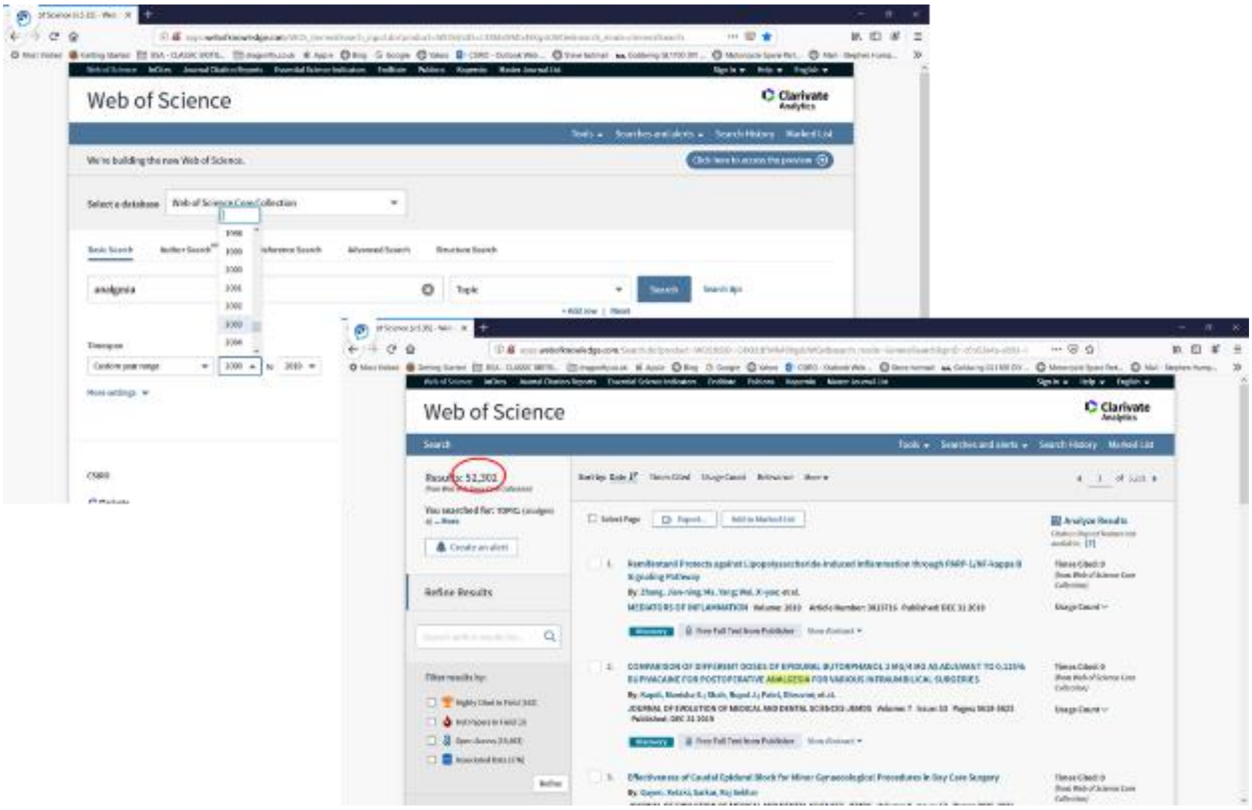

Figure 2. Refining the 'analgesia' search to 2000-2019 publications reduced the number of potential articles to 52,301.

After the primary screen against the inclusion/exclusion criteria in Table 1, each remaining abstract was read and considered in terms of 'does this article have the potential to add value to the sheep industry?' – there are many thousands of articles investigating the minutiae of pain physiology, or interaction of pharmaceutical agents with specific receptors in the body, and these were considered to be not directly relevant to the stocktake review.

Ultimately, a manageable subset of 1305 articles were included in the review. The review covered the following headings:

- Pain perception
- Assessment measures
- Husbandry procedures
- Analgesic agents
  - Local anaesthetics
  - NSAIDs
  - Sedative agents and opioids
  - New development in analgesic compounds
- Alternative analgesic modalities
  - Pulsed Electromagnetic Field (PEMF)
  - Electroacupuncture (EAP)
  - Transcutaneous Electrical Nerve Stimulation (TENS)
  - Topical cooling / cryoanalgesia
- Delivery systems
- Other knowledge gaps, e.g. neuropathic pain.

CONCLUSIONS/RECOMMENDATIONS

The review concluded that:

> *"Although a number of analgesic solutions are now available for sheep (Ilium Buccalgesic® OTM; Numnuts®; Metacam® 20; Tri-Solfen®), providing some amelioration of the acute pain responses, this review has highlighted a number of potential areas for further research, some of which can provide industry deliverables in a reasonably short time frame (within 5 years), while others are of a more long-term character."*

Some recommendations for further research include:

Activities with short term (< 5 years) outcomes

- Continue to systematically evaluate multimodal approaches to the various husbandry procedure methodologies (e.g. surgical or ischaemic) and combinations (e.g. mulesing with castration).
- Investigate non-pharmacological factors that can affect the pain response (e.g. handling, distraction or social context), toward the development of a holistic approach to integrated pain management.

Activities with medium term (5-10 years) outcomes

- Continue to develop delivery systems that are consistent, safe and easy to apply in the field.
- Continue to develop novel (e.g. sensor) technologies that allow practical assessment of pain status in a commercial setting.
- Develop formulations that allow for sustained analgesia (e.g. Combination formulations; sustained-release formulations; in-feed medication).
- Investigate enterprise-level benefit of use of analgesia for routine husbandry procedures. This data can support adoption of analgesia.
- Investigate the potential for 'natural' vanilloids (e.g. Eugenol or Camphor) and vapocoolants to be used as part of a multimodal approach.

Long term research programs

- Develop a deeper understanding of the molecular physiology associated with ischaemia-dependant procedures, such that selection of appropriate analgesic strategies to address pain associated with ring castration and tail docking.
- Investigate the potential for current and novel analgesic approaches to prevent the development of spinal pathologies associated with sustained or neuropathic pain.

FURTHER INFORMATION
A detailed final report of the project, including a response to the recommendations from AWI, is available at www.wool.com/flystrikelatest.

Dr Alison Small is a veterinarian, with PhD in veterinary public health (food microbiology). She leads livestock welfare research in a number of areas including pain mitigation for livestock; alternatives to painful husbandry procedures, humane slaughter and neonatal development and survival. Interests also in meat processing, safety, *shelf-life and quality.*
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FLYBOSS — IMPROVING FLYSTRIKE MANAGEMENT IN SHEEP

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INTRODUCTION

FlyBoss is one component of ParaBoss (ON-00382 ParaBoss Phase II), the project that delivers Australia's premier resource for parasite management of sheep and goats. FlyBoss commenced development in 2008 as part of the Sheep CRC II, later joining with WormBoss and LiceBoss under the ParaBoss umbrella in 2014, funded by AWI and MLA. FlyBoss continues to offer flystrike prevention and treatment solutions for Australian sheep producers and their advisors. The FlyBoss website is the primary repository of flystrike information and tools, and awareness of the content is strongly promoted through the ParaBoss News e-newsletter, the ParaBoss Facebook page and ParaBoss articles in Beyond the Bale, as well as other channels. During the two years since progress was reported at the 2018 AWI Flystrike RD&E Technical Update, further material has been created or is in development.

The current phase of ParaBoss has been extended until the end of June 2021. An industry strategy for ParaBoss Phase III will be developed over the next 12 months and is expected to include content targeting parasite management in cattle as well as sheep and goats.

NEW OR UPDATED FLYBOSS CONTENT

FlyBoss website

FlyBoss content on www.flyboss.com.au is relatively mature and complete, providing the majority of current information required by producers and their advisors to cost-effectively manage flystrike, improve animal welfare outcomes, and maintain low pesticide-residue wool while maintaining the effectiveness of chemical control products. The FlyBoss website contains a number of decision support tools; one of these is undergoing redevelopment and another new tool will be published in mid-2020.

The current Products Tool, which is shared with LiceBoss, provides a listing of commercially available products for flystrike (and lice) prevention and treatment. The tool is being re-developed in line with the upcoming release of ParaBoss content for cattle, expected in late 2021.

ParaBoss content is currently being updated to include parasite information relevant for cattle, and the ParaBoss Technical Committee recently reviewed the existing Products Tool for flies and lice, as well as the worms Drench Database search tool, to see what improvements or additions were needed to also address cattle. The solution, in development now, will be a combined database of all commercially available sheep, goat and cattle products for all parasites, with seven separate search tools specific for each animal-host interaction. One of these will be fly control products for sheep and will be hosted on FlyBoss. All of the search tools will be very similar so that users who become familiar with one tool will be able to rapidly use any of the others, which will collectively deal with sheep, cattle and goats (worms only), and their parasites: flies (blowflies of sheep and buffalo flies of cattle), lice, worms and ticks (cattle only).

A particular addition driven by the ParaBoss Technical Committee is to more prominently highlight resistance status of chemicals, so that users can make more informed chemical choices to help them in their quest to limit further development of pesticide resistance. The increase in pesticide resistance is seen as a major industry issue as there are relatively few new classes of chemicals in development by pharmaceutical companies. As such, it is imperative that producers are provided with the knowledge to use existing chemicals wisely so that they remain effective for as many years as possible.

FlyBoss also has two current tools that assist producers to assess the risk of flystrike during the year and to simulate management and chemical use decisions to maximise flystrike control (www.flyboss.com.au/sheep-goats/tools.php).

Two key documents have also been added to the site. These provide guides on how to manage flystrike, either to delay the development of pesticide resistance or to manage flystrike in the face of existing pesticide resistance on a particular farm. They were developed in conjunction with AWI's Sheep Blowfly Resistance Management Strategy Working Group. The site has also included ongoing promotion to encourage sample submission for the AWI project conducting flystrike pesticide resistance testing.

The FlyBoss website itself was also re-released in a responsive format in 2019. This has greatly improved ease of use for the increasing number of users who access the site via mobile and tablet devices.

Google Analytics data (Table 1) shows a steady increase in use of the FlyBoss site from 2014 to 2018, but a drop during 2019. It is expected that the severity and extent of drought conditions nationally during 2019 would have resulted in a much lower incidence of flystrike, a substantial drop in the sheep population, many fewer sheep owners (as destocking occurred) and the opportunity to cull more susceptible sheep. As a result, it is not unexpected that site usage declined.

Table 1. Google Analytics for FlyBoss website use 2014–2019.

Year	Page views	Users
2014	28,877	11,579
2015	30,881	14,395
2016	39,042	18,513
2017	46,298	22,562
2018	58,904	30,918
2019	51,236	24,961

Figure 1. Screen shot of the FlyBoss website.

ParaBoss articles in AWI *Beyond the Bale*
ParaBoss contributed four key articles to AWI's *Beyond the Bale* magazine relating to flystrike prevention and management since the 2018 AWI Flystrike RD&E Technical Update:

- Flystrike treatments during drought (March 2019)
- Resistance management strategy for the Australian sheep blowfly (*Lucilia cuprina*) (June 2019)
- A fly in the ointment (managing flystrike when there is already evidence of chemical resistance) (December 2019)
- Parasite management plans (March 2020).

A small article was also published that encouraged producers to submit samples for pesticide resistance testing through another AWI project.

ParaBoss Facebook page
The page has been operational since 2016 and can be found at: https://www.facebook.com/paraboss.com.au
Generally, about 4 posts per week are made, of which one per week is related to flystrike management and prevention. These posts are used to bring a wider audience to the FlyBoss website and the posts focus on topics

relevant to the time when published. There has been a steady increase in followers to the ParaBoss Facebook page, increasing from about 1200 followers in June 2018 to about 1800 in May 2020.

AWI Sheep Blowfly Resistance Management Strategy Working Group participation
ParaBoss staff have taken a leading role in the work undertaken by the AWI Sheep Blowfly Resistance Management Strategy Working Group. This has included drafting, editing and publishing the two articles about pesticide resistance already described above.

Training for Advisors
Two ParaBoss conference/workshops have been held since the 2018 AWI Flystrike RD&E Technical Update. A fully subscribed ParaBoss conference was held in August 2018 that 125 industry advisors and service providers attended. This included a flystrike session. The first presentation was from Brian Horton (TIAR/UTAS) who described the FlyBoss tools and the flystrike component of the AskBill tool (also developed by Brian). The second presentation was from Geoff Lindon (AWI) on Improving lifetime welfare–breech strike. This covered breeding for reduced breech strike, including national flock progress, ASBVs and the search tools on the Sheep Genetics website; the National Wool Declaration; pain relief at marking; and factors to consider when moving to a non-mulesed flock. The presentations were followed by a 25-minute open discussion forum.

The March 2020 ParaBoss workshop, held in Adelaide, was also fully booked with 60 participating advisors and assistance from 10 producers and 10 technical experts/facilitators/presenters. The event included three workshop sessions: worms, flies and lice, as well as presentation sessions. During each workshop six advisors worked with one farmer and one technical expert to create a parasite management plan for the farmer. Approximately half of the invited advisor attendees were from rural merchandise stores, with the remaining made up of private and government veterinarians, pharmaceutical company staff, university lecturers and WEC service providers.
This workshop focussed on increasing the skills of industry advisors by providing them with a process (and practice) that would enable them to quickly and easily work with their customers and clients, asking the right questions and discussing the pertinent topics, so they could create a customised and well-informed flystrike, worm and lice management plan for those clients/customers. The workshop process could be repeated in other locations in future to target more advisors.

Sheep Parasite Management certificate course
In development is a Sheep Parasite Management certificate course for industry advisors from which participants will both learn and be assessed on every core topic in WormBoss, FlyBoss and LiceBoss. It will be the most comprehensive applied sheep parasite management qualification of its type world-wide. While this course was developed to target rural merchandise staff, who play a prominent role in providing advice (particularly about treatment products) to sheep producers, it will be promoted to all industry “advisors” and be accessible to anyone. It will be operated on a user-pays basis.

The certificate has worm, fly and lice modules and is presented as a series of quizzes that assess the participants. Each quiz not only assesses, but teaches, by providing the information required. It uses a contemporary well-accepted process already familiar to farmers (such as that used for online Livestock Production Assurance and Chemical User accreditation) that allows participants to learn as they go, rather than in advance of the assessment stage.

The product will be completed by June 2021 in readiness for enrolments after this, in ParaBoss Phase III.
The success of the activity hinges around ParaBoss certifying successful graduates and heavily promoting them to industry. These graduates will be able to demonstrate an advanced knowledge of sheep parasite management and should be sought after by both producers and employers, which will provide incentive for more participants to enrol. The standard required to graduate will be high and comprehensive and therefore the qualification will be meaningful. Ultimately it will yield an increase in the quality of parasite management advice provided to producers.

Podcasts

In 2019 ParaBoss launched a series of podcasts (called Wormcasts) that included episodes relevant to flystrike prevention (www.paraboss.com.au/multimedia).

SUMMARY

ParaBoss continues to be the premier Australian source of independent, practical parasite management information, including FlyBoss for flystrike management. While FlyBoss is already mature, improvements are ongoing, ensuring this program remains a world-leading resource for the management of flystrike.

Dr Deb Maxwell is the Executive Officer of ParaBoss. With a Veterinary Science degree, plus postgraduate education and extension qualifications, she has built her extensive practical knowledge and skills in sheep management and breeding over 25 years in sheep extension with DPI Qld, the Sheep CRC and ParaBoss, and from running her own Merino stud for 22 years. Practicing what she preaches, she is a member of Sheep Genetics MERINOSELECT and her stud breeding objective is focused on breeding productive, parasite-resistant, superfine Merinos.

BENCHMARKING AUSTRALIAN SHEEP PARASITE CONTROL

AUTHOR
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SUMMARY
Sheep parasites present an enormous cost to the Australian sheep industry with the latest estimate being $715 million per year for worms, flies, lice and liver fluke (Lane *et al.* 2015). Sheep producers face a number of challenges associated with parasite control including parasite resistance to chemical actives, wool residues, occupational health and safety, animal welfare and environmental contamination. The 2019 Australian Sheep Parasite Survey was commissioned by Australian Wool Innovation Ltd (AWI) in order to provide industry with information to address sheep parasite control needs and was conducted by researchers at the University of New England, Armidale. This project summary report focuses mainly on the survey results addressing blowfly and some worm control practices. For links to additional results on worms and lice see the Further Information section below.

METHODS
This was the third survey commissioned by AWI on benchmarking parasite control with the two previous questionnaires surveying the years 2003 and 2011, allowing measurement of change in parasite incidence and control practices. The current survey, covering practices in 2018 and change over the previous 5 years, was conducted online for the first time and, also for the first time, comparisons were made between parasite control practices of wool sheep, meat sheep and cross-bred sheep (Enterprise). The survey was open for 10 weeks from 5 February 2019 and at the end of the survey period an invitation to complete a short five question survey was emailed to the same email cohort to measure non-response bias. Respondent postcode was used to allocate responses into Meat and Livestock Australia (MLA) Reporting Regions (Region).

RESULTS
Property and operation details
The number of main survey respondents was lower than in previous years (2018, n=354; 2011, n=575; 2003 n=1365). This was possibly due to survey fatigue in general, the length of the questionnaire, presentation of the survey on an online platform (respondents were most likely to access the survey after an email reminder) and severe drought over large parts of the continent. In addition, there were 250 usable responses to the short survey. The average age of respondents was 57 years.

The majority of respondents selected wool based enterprises as their chosen enterprise (67%) followed by meat sheep (21%) and cross-bred sheep (10%). The main source of income on the reporting properties came from wool sheep (41.7%) followed by meat sheep (27.6%), cattle (11.9%) and cropping (13.2%) with significant differences between Regions as expected. Figure 1 shows the location of respondents by Region. The survey year (2018) was drier than average in all Regions except for Tasmania.

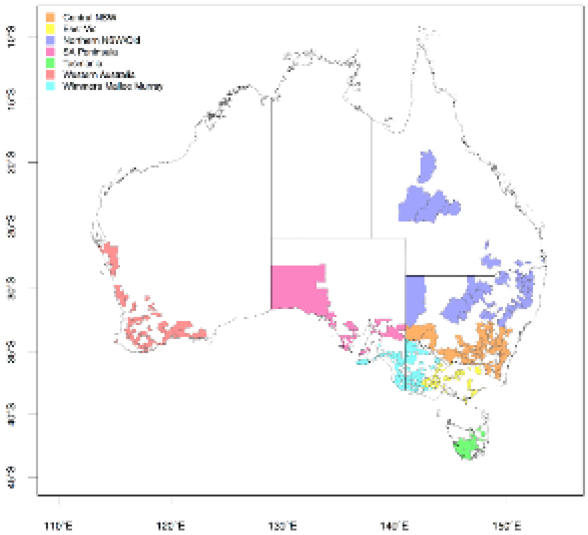

Figure 3. Location of survey respondents within MLA Reporting Regions.

Flystrike control

A lower proportion of respondents reported breech strike and body strike in their flock in 2018 compared with 2011, together with a lower incidence within the flock (Table 1). A likely factor in this reduction is that 2018 was a much drier than average year (mean reported rainfall 407mm), unlike 2011 (mean reported rainfall 650mm).

Table 1. Proportion of respondents reporting breech strike and body strike and incidence within respondent's flock in 2018 and 2011 survey years.

	Proportion respondents reporting flystrike				Incidence of flystrike in flock			
	Breech strike		Body strike		Breech strike		Body strike	
Sheep class	**2018**	**2011**	**2018**	**2011**	**2018**	**2011**	**2018**	**2011**
Adult ewes	37%	78%	14%	68%	2.7%	4.1%	2.1%	5.5%
Wethers	7%	45%	3%	49%	1.8%	5.5%	2.1%	5.7%

Respondents favoured an integrated approach to flystrike control with the most popular methods being timing of crutching (76%), timing of shearing (63%) and preventative chemical treatment (76%). Nationally, 47% used mulesing and 46% used genetic selection for flystrike control. The proportion using mulesing was lower than reported in the AWI Merino Husbandry Practices survey (63% mulesed wether lambs and 70% mulsed ewe lambs, Sloane 2018), however, that survey targeted wool producers whereas this survey included a significant proportion of meat producers (20%) who rely less on mulesing due to the lower susceptibility to blowfly strike of meat breeds. Merino x Merino producers were significantly more likely to use mulesing (69%) and genetic selection (58%) indicating an ongoing reliance on mulesing whilst the slower gains from genetically-based strategies build up. Meat x Meat producers were significantly less likely to mules (9%) or use genetic selection for flystrike control (26%). When asked specific questions on genetic selection for sheep that are less susceptible to flystrike, 56% indicated they used visual traits for selection and 13.3% of Merino producers 17% used Australian sheep breeding values (ASBVs) for ram selection, most of those who used ASBVs were Merino producers (77%).

A very high proportion of producers used pain relief with mulesing in ewe lambs (87%) and wether lambs (91%). This is a significant increase on use of pain relief in the 2011 survey (59% used in ewes lambs, 64% in wethers). Most used Tri-Solfen® (82%), 3.4% used both Tri-Solfen® and Buccalgesic® and only 0.8% used Buccalgesic® only.

When chemical treatments were given, they were predominantly given as a preventative treatment at approximately the same time every year (66%). Dicyclanil was the predominant chemical used with this method (55%) and backliner/spray the most popular method of application (66%). There was a low proportion of respondents suspecting resistance to flystrike control chemicals (5%).

The use of the FlyBoss website has increased substantially with 59% visiting the site and 18% using the site to make changes compared with 11% visiting in 2011 and 2% using the site to make changes.

Internal parasites – worms

Nearly two thirds of respondents used planned preventative treatments with 'prepare clean pastures using spelling' the second most popular method for worm control (Figure 2). There is low penetration of methods based on maintaining refugia for reducing drench resistance such as leaving some sheep un-drenched. The exception was in Western Australia where nearly 40% used that technique.

Forty percent of respondents used worm egg count (WEC) monitoring in 2018 with no effect of Region or Chosen enterprise. The mean number of WEC monitors per year was 3.1/year for ewes, lambs and weaners. The frequency of drenches was similar across sheep classes (ewes 2.1/year, lambs and weaners 2.1/year). The top three anthelmintic groups used in 2018 were macrocyclic lactones (39%), benzimidazoles (20%) and levamisole (17%), with very low uptake of the newer anthelmintic actives such as monepantel (3.2%), derquantel (3.2%) and praziquantel (0.7%). The top three drench actives used were abamectin (23.6%), levamisole (17.4%) and moxidectin (14%), these were the same top three actives reported in the 2011 survey. Most drenches were given as single actives (55%) with triple combinations the next common (21.5%).

Only 37% of respondents carried out a drench test of any kind over the 5-year period from 2014 to 2018 leaving nearly two thirds who do not know their drench resistance status.

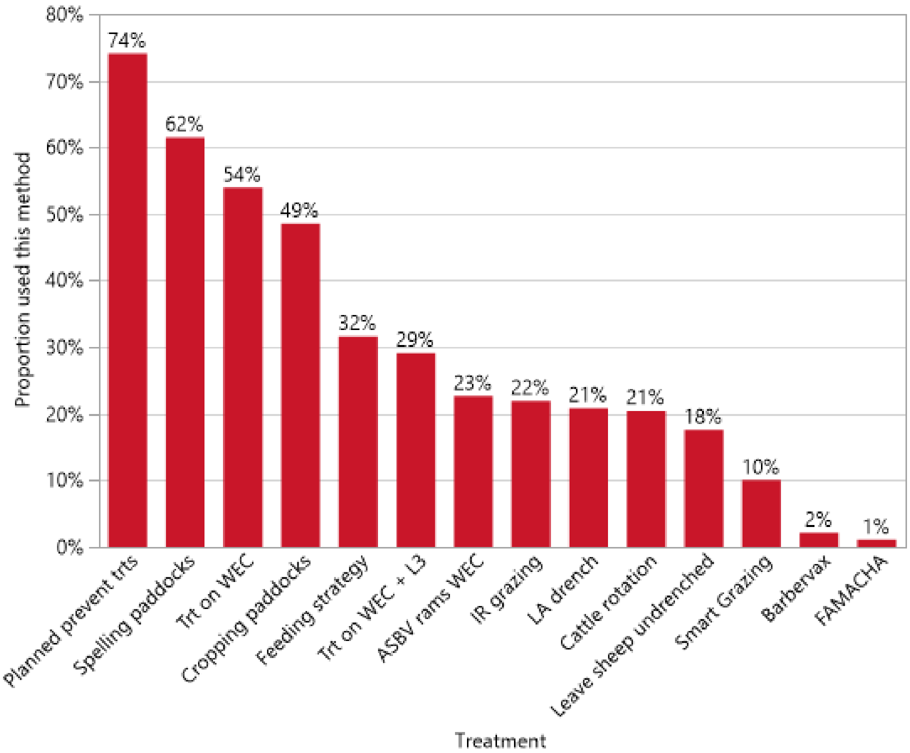

Figure 4. National proportion of respondents using a method or technique for worm control in 2018.

The use of the WormBoss website has increased significantly (63% visited site, 28% used site to make changes) since 2011 (16% visiting, 5% using the site to make changes). Over half of respondents indicated that face to face workshops were their preferred method of delivery of information (53%).

PROJECT FINDINGS – KEY MESSAGES TO WOOLGROWERS

- Merino wool producers have responded to consumer concerns regarding animal welfare and have overwhelmingly adopted the use of pain relief during mulesing and are moving towards non-mulesed sheep by adopting genetic selection to breed sheep that are less susceptible to flystrike through visual traits and ASBVs.
- Woolgrowers continue to demonstrate their adaptability through increased use of worm control methods and techniques such as grazing management, WEC monitoring and genetic selection. This has helped maintain chemical treatment frequency at relatively low and stable levels. However, when it comes to protecting drench resistance status there is more that can be done such as conducting regular drench resistance tests, basing drench decision making on the "Drench Decision Guide" in WormBoss and using combination drenches.
- The results of the Australian Sheep Parasite Survey suggest a continued need for information delivery and grower education across all major parasites. The ParaBoss suite of websites offers woolgrowers an invaluable resource for parasite control and allows instant access to up to date information. There was an encouragingly very large increase in the use of these websites including using the information to make changes to parasite control practices. However, the average age of respondents to the survey and their preference for face to face workshops indicates that that form of learning is still vitally important for dissemination of information.

FURTHER INFORMATION

- A summary of all the survey results was reported in the recent *Beyond the Bale* Issue 82, March 2020, Page 40 "Parasite control: How do you compare?".
- Further Benchmarking Australian Sheep Parasite Survey results on worms was published in the following ParaBoss newsletter feature article: http://www.wormboss.com.au/sheep-goats/news/articles/general/australian-sheep-parasite-management-survey-2019-worm-and-liver-fluke-results.php
- An article on the survey results for lice is expected to be published in the ParaBoss newsletter and on www.liceboss.com.au in mid-June.

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Dr Alison Colvin holds a Bachelor of Science in Agriculture with honours from the University of Sydney and completed her PhD at the University of New England in the use of intensive rotational grazing for worm control in sheep. She is currently a research fellow at the University of New England managing the Australian Sheep Parasite Survey for Australian Wool Innovation and mobile acquired data collection for an ACIAR project on goat production systems in Laos.

NATIONAL WOOL DECLARATION – ADOPTION, COMPLIANCE AND PREMIUMS/DISCOUNTS

AUTHOR

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SUMMARY

AWEX developed the the National Wool Declaration (NWD) for Mulesing Status in consultation with the Australian wool industry, to assist buyers and their clients gain access to information on issues that can influence purchasing decisions but cannot be measured. The NWD is voluntary, allowing growers to communicate directly with prospective buyers, processors, and retailers. The NWD Integrity Program (NWD-IP) comprises Desktop Audits and On-Farm Inspections to build pipeline confidence in Australian wool. Non Compliant Declarations can seriously reduce the credibility of the program and confidence in the Australian wool industry. AWEX has collated Mulesing Status data declared in the NWD from 2008 to YTD 30th April 2020. This report summarises this information and associated trends and provides commentary on recent changes to the NWD.

PROJECT REPORT

This report summarises the statistics related to the National Wool Declaration (NWD) and its Integrity Program (IP), namely Adoption and Compliance Rates and Premiums/Discounts for Mulesing Status. It provides an update on NWD Version 8.0, effective 1st July 2020. These definitions apply:

Mulesing: "the removal of skin from the breech and/or tail of a sheep using mulesing shears". Importantly, the removal of any skin from the breech and/or tail of a sheep is determined as mulesing.

Non Mulesed (NM): No sheep in this mob has been mulesed.

Ceased Mulesing (CM): No lambs born on this property in the last 12 months have been mulesed and no purchased ewes or wethers are mulesed.

Pain Relief (PR): All sheep in the mob were mulesed using a registered pre- or post-operative Pain Relief product.

Mulesed (M): Some/all sheep in this mob have been mulesed.

1. Adoption Rates for Seasons 2008 to 2019 (YTD 30 April 2020)

Tables 1, 3 & 5 are based on % sum of bales, all breeds and wool types, first-hand offered, P and D Certificates.

Table 1 shows that the NWD adoption rate (NM, CM, PR and M) has increased from 38% to 73% from Season 2008 to 2019, with concomitant increases in NM, CM and PR and declines in M and Not Declared (ND).

Table 1. NWD Adoption Rates (%) by Mulesing Status

Season / MS	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
NM	3	4	6	6	7	6	8	9	10	12	13	**14**
CM	3	3	3	2	2	2	3	3	3	3	3	**4**
PR	3	5	8	12	14	14	19	23	28	32	36	**38**
M	29	25	25	24	21	20	20	20	21	20	19	**18**
ND	62	63	58	55	55	57	51	45	39	34	30	**27**
Total (% Declared)	**38**	**37**	**42**	**45**	**45**	**43**	**50**	**55**	**61**	**66**	**70**	**73**
Tot Bales	1,416,656	1,467,058	1,416,656	1,467,058	1,589,514	1,456,151	1,598,009	1,452,077	1,617,165	1,487,894	1,349,198	995,359

Table 2 (Seasons 2011 to 2019) is based on % sum of bales, Merino ≤24.5 µm, all wool types, first-hand offered, P and D Certificates. It provides a comparison with Table 1, which is based on all sheep breeds and all microns.

Table 2 shows that for Merino ≤24.5 µm, the NM adoption rate doubled between Seasons 2011 and 2019. At 10%, it is less than all sheep breeds and microns (14%). Not surprisingly, since mulesing *per se* is more common in Merinos, the PR adoption rate is higher for Merino ≤24.5 µm (44%) compared with all sheep breeds (38%). There is a slightly higher overall adoption rate (NM, CM, PR and M) for Merino ≤24.5 µm (75%) compared with all sheep breeds and all microns (73%).

Table 2. NWD Adoption Rates (%) by Mulesing Status (Merino ≤24.5 µm)

Season / MS	2011	2012	2013	2014	2015	2016	2017	2018	2019
NM	5	5	5	5	6	6	8	8	**10**
CM	3	3	2	3	3	3	2	2	**4**
PR	14	17	17	22	27	32	37	42	**44**
M	26	22	21	21	21	21	20	19	**17**
ND	53	53	55	49	44	38	33	28	**25**
Total (% Declared)	**48**	**47**	**45**	**51**	**57**	**62**	**67**	**71**	**75**
Tot Bales	1,232,586	1,285,751	1,200,160	1,331,234	1,215,810	1,279,596	1,296,516	1,095,621	827,756

Table 3 shows on a state-by-state basis that the NWD adoption rate (NM, CM, PR and M) has approximately doubled for NSW, QLD, SA, TAS and VIC from 2008 to 2019. It is not clearly understood why WA continues to lag at only 43%.

Table 3. NWD Adoption Rates (%) by State

Season / State	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
NSW	40	40	43	47	50	49	56	63	68	71	75	**80**
QLD	28	25	30	29	38	35	40	47	53	65	75	**83**
SA	35	34	44	48	45	45	56	61	67	71	77	**80**
TAS	48	47	69	55	55	47	51	67	80	81	82	**84**
VIC	43	43	45	50	51	51	61	65	76	79	82	**84**
WA	33	27	37	35	28	24	21	24	31	34	40	**43**

The data in Table 4 (Seasons 2011 to 2019) is based on % sum of bales, Merino ≤24.5 µm, all wool types, first-hand offered, P and D Certificates. It provides a comparison with Table 3, which is based on all sheep breeds and all microns. In general, for NSW, SA, TAS and VIC, there is a slightly higher adoption rate amongst Merino woolgrowers, compared with all sheep breeds and all microns. This is confirmed in Table 5, where the NM adoption rate for <18.6 µm is 35%, for 18.6 – 20.5 µm is 17%, reducing to 6% for 20.6 - 24.5 µm.

Table 4. NWD Adoption Rates (%) by State (Merino ≤24.5 µm)

Season / State	2011	2012	2013	2014	2015	2016	2017	2018	2019
NSW	51	53	53	60	67	72	75	79	**84**
QLD	30	39	36	40	48	53	65	75	**83**
SA	50	47	47	58	62	68	73	80	**84**
TAS	57	59	50	53	70	84	85	86	**88**
VIC	56	56	55	66	70	80	83	86	**88**
WA	35	28	24	21	24	31	35	41	**44**

Table 5 shows variation in the adoption rates for 5-micron categories. Wool <18.6 µm has a relatively high proportion of NM wool and a corresponding high level of CM compared with other microns indicating their transition away from mulesing. This is likely due to the strong connection these growers have with their market, through for example, the ASWGA. Wool >24.5 µm has a similar NM adoption rate, but it could be higher as it is likely that many of these growers do not mules. From the bottom row, the adoption rate generally declines as the micron gets coarser, indicating a lack of understanding of the importance of the NWD for all end-uses.

Table 5. NWD Adoption Rates (%) by Micron Category

Season	<18.6 µm		18.6 – 20.5 µm		20.6 – 22.5 µm		22.5 – 24.5 µm		>24.5 µm		Total	
MS	No. Bales	%	No. Bales	%	No. Bales	%	No. Bales	%	No. Bales	%	No. Bales	%
NM	49,255	35	24,000	17	7,786	6	8,725	6	50,317	36	140,083	100
CM	17,054	48	9,078	26	2,939	8	594	2	5,556	16	35,221	100
PR	171,382	45	149,076	40	36,765	10	4,433	1	15,215	4	376,871	100
M	54,294	31	63,335	36	23,250	13	5,108	3	28,454	16	174,441	100
ND	79,319	30	98,209	37	28,862	11	12,366	5	49,987	19	268,743	100
Total (% Declared)	**371,304**	**29**	**343,698**	**25**	**99,602**	**7**	**31,226**	**2**	**149,529**	**10**	**995,359**	**73**

2. NWD-IP Compliance Rates for Seasons 2010 to 2019 (YTD 30 April 2020)

The NWD-IP commenced in 2010 and comprises: Desk Audits to validate that the correct Mulesing Status is published , with corrections made prior to sale (~1,000 p/a); Verifications of the use of PR at mulesing (~350 p/a); and On-Farm Inspections to confirm NM/CM status (~225 p/a). The number of Desk Audits and PR Verifications was increased in March 2020 when Inspections were put on hold due to COVID-19.

The Desk Audit compliance rates are presented in Table 6. A main factor contributing to Non Compliance for Desk Audits is the incorrect completion of CM. The definition of CM changed to two parts in July 2017 (see 1. above). Growers must fulfil <u>both</u> requirements, not just one of them, with Non Compliance usually occurring because they have purchased Mulesed or PR ewes or wethers.

Table 6. NWD-IP: Compliance Rates for Desk Audits (NM, CM and PR)

Season	No. Desk Audits	No. Compliant	% Compliant
2010	781	515	**66**
2011	944	650	**69**
2012	1362	882	**65**
2013	984	571	**58**
2014	981	665	**68**
2015	846	623	**73**
2016	1115	859	**77**
2017	907	855	**94**
2018	1061	851	**80**
2019	1112	907	**82**

The compliance rate for PR Verifications is currently ~95% (see Table 7). Non Compliance generally occurs for two main reasons (a) the grower purchases sheep and assumes that they were mulesed with PR - the mulesing status of any purchased mob must be able to be verified (e.g. evidence from the vendor) or (b) the classer or other person assumes the mobs were mulesed with PR and the growers signs the NWD without checking – the NWD is the grower's responsibility and it should be checked prior to dispatch to the broker.

Victoria has become the first state in Australia to mandate Pain Relief for mulesing and it is expected that the declaration rate of PR will increase.

Table 7. NWD-IP: Compliance Rates for PR Verifications

Season	No. Verifications	No. Compliant	% Compliant
2010	100	88	**88**
2011	100	91	**91**
2012	100	89	**89**
2013	100	90	**90**
2014	100	76	**76**
2015	116	102	**81**
2016	197	184	**93**
2017	247	227	**92**
2018	326	312	**96**
2019	278	265	**95**

The compliance rates for NM/CM Inspections over the past decade is around 85% (see Table 8). Non Compliance generally occurs for two main reasons (a) the grower purchases sheep and believes they are NM (this applies to both Merinos and Crossbreds) – the buyer should check the mulesing status at the time of purchase and verify NM status by checking for scars on the breech and along the tail or (b) the grower forgets when he/she ceased mulesing and older mulesed sheep are found in the mob.

Table 8. NWD-IP: Compliance Rates for NM/CM On-Farm Inspections

Season	No. Inspections	No. Compliant	% Compliant
2010	245	206	**84**
2011	229	183	**80**
2012	277	240	**87**
2013	237	201	**85**
2014	257	211	**82**
2015	237	204	**86**
2016	210	187	**89**
2017	198	166	**83**
2018	221	190	**86**
2019*	62	58	**93**

*On-Farm inspections hampered by on-going drought and a temporary halt due to COVID-19.

3. Mulesing Status Premiums and Discounts for Seasons 2013 – 2019 (YTD 30 April 2020)

The calculation of Premiums and Discounts (c/kg clean) for Mulesing Status requires that as many criteria as possible can be held constant. The following describes the dataset used:

- Australian Stored; Merino Fleece/Weaners and Crossbred Fleece
- >30 N/ktex, >60% Schlum Dry Yield, <2.2 VMB, Styles 4/5, Good/light colour (incl. H1), P Certificate
- Lengths according to Diameter Range: 70–95 mm (<18 µm), 75–99 mm (19–21 µm), 83–104 mm (22–24 µm), 90–110 mm (26–29 µm), 100–130 mm (30–34 µm)
- Records per group (micron/NWD status) >2, empty cells when not enough data to generate a P or D.
- Comparison with prices for wool declared as Mulesed.

From Table 9, Season 19 sees the continuation of Premiums for NM wool, ranging from 78 c/kg clean for 16 µm wool to 16 c/kg for higher micron Non-Merino wool. With variation around these mean values, some wools will achieve higher premiums and some lower or non-existent. Premiums for CM status are evident but less strong compared with NM. The values recorded for PR and ND may not be significant.

Table 9. Premiums and Discounts for Mulesing Status

		MERINO							NON-MERINO			
		16	**17**	**18**	**19**	**20**	**21**	**22**	**27**	**28**	**29**	**30**
NON MULESED												
Season	**2013**	16	12	9	9	4	-3	2	0	-1	4	16
	2014	28	15	11	12	13	0	5	3	4	-3	28
	2015	10	15	7	8	8	1	-15	0	-1	1	4
	2016	14	15	30	13	21	18	-12	-1	11	-5	-1
	2017	36	44	57	44	36	18	-13	20	6	1	2
	2018	47	53	46	38	20			12	8	7	1
	2019	**79**	**43**	**46**	**39**	**34**	**28**	**34**		**16**	**16**	
CEASED MULESING												
Season	**2013**	4	14	9	-2	3	1	16	2	-4	4	4
	2014	11	4	-3	10	-5	-3	17	-1	5	-3	11
	2015	51	15	3	8	5	4	-1	2	5	-8	-3
	2016	1	21	15	5	5	3	8	30	9	-20	20
	2017		68	8	19	4	0	-10	-8	4	-5	5
	2018		53	21	-1	3				15		9
	2019	**37**	**32**	**37**	**-8**	**3**	**12**			**3**	**4**	
MULESED with PAIN RELIEF												
Season	**2013**	0	2	0	3	0	3	-15	16	-9		0
	2014	11	6	-2	1	-1	-4	1	3	0	-1	11
	2015	2	2	-4	0	1	0	2	8		9	14
	2016	6	-1	6	1	3	4	0	14	19	-11	12
	2017	24	4	5	8	1	3	1	27	6	-2	-1
	2018		3	0	2	3	2	-2	0	13	23	
	2019	**18**	**14**	**12**	**0**	**0**	**-1**	**8**	**22**	**0**	**-3**	
NOT DECLARED												
Season	**2013**	-2	2	-1	0	0	0	-4	-4	3	-3	-2
	2014	5	4	-4	0	-1	-4	-2	-4	0	-2	5
	2015	8	5	-2	-4	-4	0	0	-6	-4	-2	-2
	2016	-1	6	1	-12	-7	1	-3	1	0	-2	1
	2017		6	11	-9	-4	-2	-9	-8	-2	-9	0
	2018		2	-4	-8	-2	-5	-3	-21	-8	-10	-18
	2019	**-8**	**-3**	**-1**	**-5**	**-3**	**-3**	**-12**	**-9**	**-12**	**-5**	

4. NWD V8.0 – Recent Changes

The Review resulting in NWD V8.0 has just been finalised with changes to be released in data systems on 1st July 2020. The Review received many submissions from Australia and overseas, including downstream stakeholders such as retailers. All comments/views were constructive and demonstrated a diversity of opinion.

One focus was whether and/or how alternative methods to mulesing should be recognised in the NWD. At the time, clear and concise analysis of the use of Sheep Freeze Branding (or other innovation) had not been conducted. To address this situation, a specific review on this topic will be undertaken before the end of 2020, or when the results of the scheduled Animal Welfare trials have been released, whichever is sooner. Submissions will be sought from all industry stakeholders prior to this review.
The accepted recommendations for inclusion in NWD V8.0 are:

- The definitions of Mulesing (M) and Non Mulesed (NM) remain the same.
- The definition of Ceased Mulesing (CM) remains unchanged. However, it is now formatted as two separate questions to improve clarity and reduce errors.

- Pain Relief (PR) to be replaced with Analgesic and/or Anaesthetic (AA), where a registered APVMA pre- and/or post-operative product is used.

For further information on the NWD and its Integrity Program go to awex.com.au or contact the authors.

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INDUSTRY EDUCATION EXTENSION AND PROMOTION

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OVERVIEW
One of the pillars of the Flystrike Research, Development, Education, Extension and Communication Strategy 2019/20 to 2024/25 (the Strategy) is Education, Extension and Promotion, with the aim of "adoption of best practice strategies to improve lifetime welfare of sheep, reduce reliance on mulesing and support transparency in the supply chain".

Australian woolgrowers operate under a wide range of sheep type, environment and farm business priorities, meaning they have adopted a complex integrated pest management approach to reducing the risk of flystrike to ensure the best lifetime welfare of their sheep. Key flystrike management tools along with mulesing include the strategic timing of crutching and shearing, accelerated shearing, judicious use of flystrike prevention chemical treatments, worm control, breeding for reduced breech wrinkle, dags, stain, breech cover, fleece rot and horns, and managing for optimal lambing dates and the lambing period to support improved management. A reasonable number of older woolgrowers managed Merinos prior to the adoption of mulesing in the 1960s and 70s using these same tools, while a few never adopted mulesing.

Moving to a non-mulesed enterprise involves rebalancing these tools. However, as improving the lifetime welfare of sheep has been a long-time, heartfelt goal for all woolgrowers, many continue to strongly believe that mulesing remains the most effective tool for achieving that vision.

Woolgrower confidence in returning to a non-mulesed Merino enterprise requires evidence and confidence on-farm in the existing tools and along the supply chain regarding improved lifetime welfare, sustainability, demand and business viability.

Influences on-farm and across the supply chain since mulesing was slowly but widely adopted that will impact on woolgrower decisions whether to continue to mules or not, include: confidence in the emerging Merino types that are increasingly productive and naturally resistant to flystrike; use of long-acting worm and flystrike control chemicals (including more recent concerns over the length of protection they offer); managing the much higher stocking rates and dag following the introduction of improved pastures and fertilisers in the 1960s; the lower levels of on-farm labour; the increasing supply chain requests for transparency regarding on-farm husbandry and animal welfare practices and balancing the mixed messages from price premiums on offer for non-mulesed wool and price discounts for unmulesed sheep.

With all this in mind, AWI produces education, training and extension resources that provide best practice information and support for woolgrowers and their advisors to meet a wide range of best practice management options for improved lifetime welfare of sheep. Some examples of these follow.

1. Develop and implement education, training and extension strategies to improve lifetime welfare of sheep
   Deliverables in this area cover a range of activities, including:
   - AWI Extension Networks (Leading Sheep Qld; Sheep Connect NSW; Best Wool Best Lamb VIC; Sheep Connect TAS, Sheep Connect SA, The Sheep's Back WA) to deliver training programs and events for woolgrowers at a regional level www.wool.com/people/awi-grower-networks
   - Lifetime Ewe Management training program www.wool.com/people/education-and-leadership/lifetime-ewe-management
   - ParaBoss (including FlyBoss, WormBoss and LiceBoss) www.paraboss.com.au
   - Merino Lifetime Productivity Project www.wool.com/sheep/genetics/merino-lifetime-productivity
   - Control of feral animals including wild dogs, feral pigs, rabbits and foxes www.wool.com/sheep/pest-animals
   - Information on managing footrot www.wool.com/sheep/welfare/footrot
   - Making More From Sheep www.makingmorefromsheep.com.au
   - Sheep Classing Workshops www.wool.com/globalassets/wool/people/education-and-leadership/stockmanship-and-merino-visual-classing-workshop-material/an-introduction-to-merino-visual-classing.pdf
   - Introduction to Stockmanship Training www.wool.com/people/education-and-leadership/stockmanship-and-merino-visual-classing-workshop-material
   - Training in best practice shearing www.wool.com/people/shearing-and-woolhandling
   - AWI Practical Workshops www.wool.com/people/education-and-leadership/practical-workshops
     - Picking Performer Ewes
     - Winning With Weaners
     - Ramping Up Repro
     - Foundations of Sheep and Wool Production
   - Regular articles in the AWI Beyond the Bale magazine www.wool.com/about-awi/media-resources/beyond-the-bale
   - Project Newsletters
   - MERINOSELECT www.sheepgenetics.org.au
   - Resources on the AWI Website (including under www.wool.com/sheep/welfare/breech-flystrike):
     - Project updates and final project reports (including on Vaccines, Genomics, Flystrike Causal Web, Rate of Genetic Gain, Insecticide Resistance)
     - Information on options for best practice analgesia and anaesthesia (Tri-Solfen, Buccalgesic, Metacam, Numnuts)
     - Plan Prepare Conduct Best Practice Lamb Marking Training Guide
     - Dealing with Dags
     - Planning for a Non Mulesed Merino Enterprise
     - Past RD&E Technical Updates (2008, 2010, 2012, 2014, 2016 and 2018)
     - Australian Animal Welfare Standards and Guidelines for Sheep
     - Managing Breech Flystrike Manual
   - AWI Social Media Channels including The Yarn podcast www.wool.com/about-awi/media-resources/the-yarn-podcast and Facebook www.facebook.com/AustralianWoolInnovation.

Course content for AWI Flystrike Workshops is currently being reviewed and updated and communication and extension resources from current or recently completed R&D projects are continually under development.

2. Monitor, evaluate and improve the success of education, training and extension activities
` Key activities include:
   - On-going woolgrower surveys
     - 2017 AWI Merino Husbandry Survey
     - 2019 Benchmarking Australian Sheep Parasite Control Survey (following from 2012 and 2007)
     - AWI Strategic Plan Monitoring and Evaluation Surveys

- AWI/MLA Wool and Lamb Forecasting Surveys
- AWI Shareholder Surveys
- AWEX National Wool Declaration; rates of adoption, premiums and discounts.
- Consultation via the AWI Woolgrower Consultation Panel and larger AWI Woolgrower Consultation Group
- Consultation with range of Breeder Groups and Associations
- AWI AVA and Genetic Reviews of the Strategy.

3. Engage with woolgrower advisors on the Strategy
   Key activities include:
   - AWI Wool Broker Forums
   - Biennial AWI Flystrike RD&E Forum and website
   - ParaBoss Technical Committee
   - Sheep Genetics.

4. Ongoing engagement with domestic and international stakeholders to ensure they understand best practice management of flystrike and the welfare implications
   The key activities include:
   - AWI AVA and Genetic Review of the Flystrike Strategy
   - AWI Animal Welfare Forum
   - Biennial AWI Flystrike RD&E Forum and website
   - Regular engagement with international and domestic brands and retailers by the Woolmark Regional Managers, both within Australia and internationally.

   Further information on AWI's engagement across the supply chain is available in the following presentation from the 2018 Flystrike Prevention RD&E Update (www.wool.com/sheep/welfare/breech-flystrike/latest-research/past-flystrike-prevention-rde-updates/#rde-market).

Geoff Lindon, AWI Program Manager Genetics and Animal Welfare Advocacy, attended Roseworthy Agricultural College for 3 years and completed a Postgraduate Diploma in Financial Management with the University of New England in 1980. He worked for 10 years on 3 parent Merino Studs, 'Bungaree', 'Egelabra' and 'Boonoke'/'Wanganella'.

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