Final report

Research and adoption pathways to provide new options for flystrike control

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Abstract

This project aimed to conduct an extensive review of past research on flystrike, with a view to highlighting areas that hold promise for providing long term flystrike control. We aimed to highlight areas where the application of modern scientific and technological advances may be able to provide increased impetus to some novel, as well as some previously explored means to control flystrike. We provide several recommendations for research activities into aspects of flystrike control: insecticide resistance management, novel chemical and biological control agents, novel delivery methods for therapeutics, improved breeding indices for flystrike-related traits, and prevention of gastrointestinal nematode-induced scouring. We also identify areas where advances can be made in flystrike control through the greater adoption of well-recognised existing management approaches: optimal insecticide-use patterns, increased use of flystrike-related ASBVs, management practices to prevent scouring. We indicate that breeding efforts should be primarily focussed on the adoption and improvement of currently available breeding tools and towards the future integration of genomic selection methods. We describe aspects of the relationship between animal health companies and the sheep industry that will impact on the ongoing availability of insecticides for flystrike control, and highlight the importance of coordinating research activities into novel therapeutics with the animal health companies who will be responsible for bringing new products to the sheep industry.

Executive summary

Background

Flystrike is a serious financial and animal welfare issue for the sheep industry. Concerns about some of the current flystrike control measures, particularly mulesing, are affecting the marketability of sheep products and the 'social licence' for sheep production. The phasing-out of mulesing, and the likelihood that drug resistance may impact over coming years on the efficacy of the currently-used insecticides, highlight the need to identify long-term flystrike control options.

Objectives

A great deal of scientific research has been conducted over many years with the aim of controlling the sheep blowfly. While this has been successful to some extent, however, long-term solutions to the problem of flystrike are still required. This project aimed to examine several proposed intervention strategies for the control of flystrike in order to provide recommendations on potential pathways for the sheep industry to be able to deal with this issue.

Methodology

We examined past research efforts on different flystrike controls, with a view to judging whether these may offer promise for long-term flystrike control. We looked at whether modern technological advances may provide new impetus to these proposed control strategies.

Results/key findings

We highlight areas that we consider to be worthy of attention, as well as those that we consider to show less promise for providing practical and impactful flystrike control options. The areas recommended for greater attention fall into two categories according to whether they are at a stage requiring substantial research input, or whether they are at a stage where a significant level of knowledge already exists, such that the emphasis now should be on greater adoption by the industry.

Research areas identified include:

- drug resistance management (drug resistance diagnostics, modelling of drug-use strategies)
- chemical and biological therapeutics
- novel delivery methods for chemical and biological agents
- development of more readily-measurable breeding indices for flystrike-related traits
- development of genomic selection methods
- prevention of gastrointestinal nematode-induced scouring.

Areas where advances can be made in flystrike control through the greater adoption of well-recognised management approaches include:

- optimal drug-use practices (resistance management strategies)
- guidelines for breeders on how to best use current flystrike-related ASBVs
- management practices (including breeding and optimal anthelmintic use) to prevent scouring.

We highlight the position of the sheep ecto-parasite drug market in the commercial priorities of animal health companies as an important determinant of progress in delivering new therapeutics for flystrike control, and the importance of coordinating research activities into novel therapeutics with the companies. We suggest that the outcome of research presently underway on flystrike vaccination should direct further investment in this area, with appropriate consideration to be given

to the biological factors that make such a strategy difficult to achieve. We highlight areas where the availability of the blowfly genome could potentially provide new impetus to developing intervention strategies, including in the areas of drug-resistance diagnostics, new chemicals, vaccination, and genetic manipulation of blowfly populations. However, we also highlight the fact that commercial and feasibility considerations will act to temper the potential for the genome to act as the basis for providing practical control options in some of these areas.

Benefits to industry

The project's recommendations describe research and adoption pathways that will be important in delivering flystrike control options to the sheep industry.

Future research and recommendations

As described above, in the present report we have highlighted research and adoption pathways that could have significant impact in increasing the ability of the sheep industry to deal with the issue of flystrike. We have highlighted several areas that warrant research investment, and which will therefore take some time to have an industry impact. We also highlight areas where the emphasis should be on greater adoption of existing technology and know-how, that can have an impact in the short term.

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1. Background

A great deal of scientific research has been conducted over many years with the aim of controlling the sheep flystrike. This has significantly enhanced our understanding of sheep blowfly biology, the causes of strike and the factors underlying susceptibility in sheep and led to a range of advances in control methods. However, new sustainable enduring solutions to the problem of flystrike are clearly required. In addition, mulesing, one of the keystone methods of control over many years and in many ways a product of this research, is no longer a tenable method of control. Hence, flystrike remains a serious problem, and concerns about some current control methods affecting the marketability of sheep products and the 'social licence' for sheep production into the future have renewed pressure for the development of more effective and efficient methods of control.

There have been many very significant scientific advances over the last few years, for example in various aspects of molecular biology, gene editing, nano-scale technology, remote sensing and machine learning that can perhaps be utilised to address difficult biological issues, such as finding a solution to the problem of flystrike. The recent sequencing of the blowfly genome is a particular advance that has opened up new possibilities for sheep blowfly control and new optimism for previously-tested approaches such as vaccines and sterile fly release. The present project reviews past research on flystrike control to determine if the application of modern scientific advances can provide new impetus to these research ideas. The project also looks at whether completely new research pathways are now possible in the modern scientific environment. Recommendations are developed for future use in a program to provide long term solutions to the problem of flystrike.

2. Objectives

1. Complete an extensive literature review of research performed in the past on the control of the sheep blowfly, including work in areas of: insecticides, vaccines, biological control and 'natural' compounds, genetic control, breeding for resistance, new formulation methodologies, scouring and repellents. The literature review will include analysis of the rationale behind each approach, the record of success or failure, and the lessons learnt from past failures.

2. An assessment of how modern scientific advances may be brought to bear on the problem of flystrike. This will involve descriptions of how these advances may be utilised to revisit some of the flystrike control methods examined in the past (from section 1, above), as well as an examination of completely new methods that scientific advances have now made possible.

3. A full assessment of each of the identified options in terms of their likelihood of success, approximate scale of each research program, approximate research program timelines, scale of their potential benefit for the sheep industry, and identification of the relevant expertise in Australia and internationally that would be most able to accomplish the research goals.

4. Recommendations on the areas of research that offer the most promise for providing means for the sustainable control of flystrike.

3. Methodology

We have performed a thorough review of past efforts to control the sheep blowfly and assessed their potential to provide long-term solutions to the issue of flystrike. We have looked at the potential for the application of modern scientific technologies to provide new options for flystrike control.

We have examined a number of potential strategies for blowfly control:

- insecticide discovery and resistance
- repellents
- biological control
- novel delivery of flystrike prophylactics and therapeutics
- vaccination against flystrike
- vaccination against fleece rot
- the prevention of scouring
- breeding for resistance
- trapping
- forecasting and detection
- genetic manipulation of the fly population.

4. Results

4.1 Insecticide discovery and resistance / blowfly genome

4.1.1 Insecticides and resistance

Control of sheep flystrike has relied on the application of insecticides for many years. These insecticides have been applied most commonly as prophylactic treatments, given in advance of expected conditions that would be favourable for flystrike (moisture, heat, humidity). Treatments to kill maggots present in existing infections (dressing treatments) have also been used.

The current mainstay of fly control is the product CLiK (sold by Elanco), with dicyclanil as its active agent. Other dicyclanil-based products are also available, sold by Zoetis and Jurox. Dicyclanil offers much longer periods of protection from flystrike compared to the other blowfly control chemicals currently available. It is closely related to cyromazine, which was the principal blowfly control chemical before the introduction of dicyclanil, and continues to be widely used. Currently there are cyromazine-based products sold by a number of companies, including Elanco, Jurox, Abbey laboratories and Norbrook. In addition, there are a number of products with the active agents ivermectin, spinosad, or imidacloprid. A recent review of sheep parasite practices in the Australian sheep industry found the most commonly chemical used was dicyclanil (40% of producers) followed by cyromazine (24%), ivermectin (12%) and spinosad (12%) and imidacloprid (4%) (imidacloprid was only newly registered for flystrike control at the time), indicating a high industry reliance on two compounds, dicyclanil and cyromazine. Cyromazine and dicyclanil are only suitable for prophylactic treatments as they are not active against the more advanced maggot life stages. The other chemicals can be used as prophylactics as well as for the treatment of maggots in existing infections (therapeutic use), although the period of protection given by spinosad is much less than for the other chemicals (< 6 weeks compared to > 10 weeks). Various products containing organophosphorous compounds (e.g. diazinon) are also available for treating existing strikes.

The sheep blowfly has shown an ability to develop resistance to chemicals used to control it in the past. This resistance has led to some compound classes no longer being effective as prophylactic treatments (organochlorines, carbamates, organophosphates and benzoyl phenyl ureas). The organophosphates remain on the market as dressing treatments for struck sheep. Low level resistance to diflubenzuron (a benzoyl phenyl urea compound) appeared soon after its introduction for flystrike control in the early 1990s. Within several more years a high level of resistance became widespread in field strains, leading to the withdrawal of claims for flystrike control for compounds in this chemical class in the mid 2000s.

Cyromazine was used widely in the sheep industry for over 30 years before resistance was first reported by Levot (2012). This period of use before the emergence of resistance was quite remarkable given its heavy use and the previously-observed emergence of resistance to some of the earlier drug groups over much shorter time intervals. Levot (2012) reported that larvae recovered from a property in southern NSW were able to develop in vitro in the presence of higher concentrations of cyromazine than a reference susceptible strain, indicating the presence of a low level of resistance. There was also a low level of resistance to dicycanil demonstrated in the in vitro assays. Baker et al. (2014) subsequently showed that thorough application of cyromazine- and dicyclanil-based products at the label's recommended doses resulted in effective control of blowflies on this property for periods consistent with the registered label claims. Levot (2013) also found that the low level of *in vitro* resistance was present in fly samples from a number of properties

neighbouring the original resistant fly collection site. Furthermore, the resistance was present in the following season in populations that survived overwintering, suggesting that there was no significant fitness deficit associated with the resistant strain. This low level of resistance to cyromazine was detected in 36 out of 58 field-collected samples across several Australian states in 2012-2014 (=62%) (Levot (2014a), with low levels of *in vitro* dicyclanil resistance also detected in 8 of these 36 cyromazine-resistant samples. As cyromazine and dicyclanil are thought to have the same (or similar) mode of action against blowfly larvae, it may not be surprising that resistance to cyromazine also results in some cross-resistance to dicyclanil. The lower impact of the resistance on dicyclanil in *in vitro* assays may be due to the greater potency of this compound compared to cyromazine. Sandeman et al. (2014) described low level of cyromazine resistance as being 'quite common'. Hence, at this point in time, 2014, it was apparent that field fly populations were starting to show a low level of resistance to cyromazine (and dicyclanil to a lesser extent) that was detectable in *in vitro* assays, but with no evidence that it was having an impact on protection periods on sheep.

The original cyromazine-resistant strain was exposed to selection pressure with cyromazine over 13 generations in the laboratory (Levot 2013). This resulted in a population showing higher levels of resistance in the *in vitro* assay; the *in vitro* resistance factor towards cyromazine increased from 2.3-fold in the original field-collected strain to 8.1-fold in the selected strain. Larval implant trials with this selected strain showed that protection periods were reduced to time periods much less than the label claims: reduced from 14 weeks to < 8 weeks for cyromazine, and reduced from 18-24 to < 11 weeks for dicyclanil (Levot 2014b). This result was based on a laboratory-selected strain, and hence was not a direct reflection of current field blowfly populations at the time of the study. However, the study demonstrated the potential for the resistance that existed in field strains at that time to have an effect on protection on sheep if it increased to higher levels over time through further intensive use of the drug in the field.

A recent AWI-funded project, led by Narelle Sales (NSW DPI), has measured *in vitro* insecticide resistance in blowfly strains submitted by graziers from regions across Australia (Sales 2020). The project provided an update of resistance towards all the currently-used insecticides, and allowed a comparison to earlier studies performed in the same laboratory. The report suggested that resistance to dicyclanil and cyromazine may already be widespread. All flocks tested from NSW (n=55) showed resistance to both cyromazine and dicyclanil (as identified by an ability of larvae to survive a 'susceptible discriminating concentration', SDC, in the *in vitro* assay), while in Victoria (n=11), 91% were resistant to cyromazine and 82% were resistant to both cyromazine and dicyclanil. Resistance was also present in West Australia and South Australia, but at lower prevalence. While these flocks were not selected randomly, and, hence, the results do not represent the real industry prevalence, the results suggest that resistance may be concerningly widespread. A comparison with an earlier study showed that the percentage of cyromazine-resistant strains had increased from 62 % (n=58) to 88 % (n=100) over the last 4-6 years. Dicyclanil resistance was found to have increased from 13.8 % to 73 % over this period.

Sales (2020) and Sales et al. (2020) also reported on an *in vivo* trial with resistant strains composed of flies derived from individuals that had survived the initial SDC assays, hence representing a mixture of resistant flies from various geographical locations. Significantly reduced protection periods for cyromazine and dicyclanil against resistant blowfly strains were demonstrated in this *in vivo* larval implant trial. Three dicyclanil-based spray-on products provided protection periods significantly less than the maximum levels described on the product labels: strikes were observed (that is, 'protection failures' occurred) at weeks 3, 4 and 9 for products with protection period claims of up to 11, 24 and 29 weeks, respectively. Protection failures were recorded after 7 weeks for a

cyromazine-based product with a label claim of up to 14 weeks protection. These results are of concern for the Australian sheep industry as it relies to a large extent on the effectiveness of preventative flystrike chemical treatments.

However, as with the earlier study of Levot (2014b), this recent *in vivo* trial used two strains of blowflies that had been exposed to laboratory selection-pressure with dicyclanil prior to the implant trial. In this way, the study provides a clear illustration of the potential impact of insecticide resistance on protection periods ('worst case scenario'), rather than an indication of the protection periods likely to be obtained against blowfly populations as they currently exist in the field. The blowfly strains were selected for 3 generations with dicyclanil prior to the commencement of the study, and then for a further 7 generations during the study. While the effect of the initial 3 generations of selection on resistance levels were not reported, the subsequent 7 generations of pressure led to an increase in resistance factors from 13.4 to 32.5 fold in the strain selected at each generation with a 'low' drug concentration, and from 25.4 to 46.5-fold in the strain selected with a higher drug concentration. Given that Levot (2013) suggested that the observed increase in resistance to cyromazine following their drug selection pressure may have occurred after only one or two generations of selection, the levels of resistance reported by Sales et al. (2020) after 3 generations of selection (13.4 and 25.4-fold in the two strains) may have been significantly higher than the levels that would have existed when the flies were initially recovered from the field. The levels at the end of the trial (after 10 generations of selection) were certainly higher than would have been the case when the flies were first recovered from the field. It is apparent, therefore, that the population of flies used in this study was more resistant (in terms of *in vitro* bioassay IC_{50} values) than would have been the case when originally collected in the field.

Hence, while Levot (2014b) and Sales et al. (2020) certainly show the potential for significant levels of drug resistance in blowflies to reduce the protection periods of cyromazine- and dicyclanil-based products, the use of laboratory-pressured strains in these studies means that the impact of current field resistances on product protection periods remains unclear. We suggest that there is a need to perform implant trials using blowfly strains after isolation from the field without imposing any further drug-selection pressure, in order to better understand the actual impact of current field resistance levels on product protection periods.

The reports of Sales (2020) and Sales et al. (2020), highlighting the increasing field prevalence of blowfly strains showing resistance to cyromazine and dicyclanil in *in vitro* assays, and the potential impact of this on protection periods, are likely to raise concerns in the industry as dicyclanil is presently the mainstay of blowfly control. While alternative chemicals are available (ivermectin, imidacloprid, spinosad) none of these provide the length of protection of some of the dicyclanil-based products (12-14 weeks compared to up to 29 weeks).

Levot and Sales (2008) described low levels of resistance to ivermectin in *in vitro* assays with diflubenzuron-resistant larvae. The recent report of Sales et al. (2020) suggested that a period of protection of only 8 weeks was observed for ivermectin against the dicyclanil-resistant strains, compared to the 12 weeks protection observed with a susceptible strain (in agreement with the product label claims), although this requires confirmatory studies.

A further concern is that resistance to macrocyclic lactone drugs (the chemical class to which ivermectin belongs), imidacloprid and spinosad has arisen in other insect species and ticks: macrocyclic lactones, Che et al. 2015, Pu et al. 2010, Klafke et al. 2017, Amanzougaghene et al. 2020; imidacloprid, Bass et al. 2015; Spinosad, Grant et al. 2019, Campos et al. 2015. Hence, resistance in the sheep blowfly to these chemicals may be expected to also emerge in the field in the future.

4.1.2 Historical role of animal health companies

Bringing new chemicals to the blowfly control market has been in the hands of the major chemical companies. Most of the chemicals were developed within the companies for the control of pests other than blowflies, and then were subsequently developed for use in blowfly control; this applies to the organophosphates (e.g. diazinon), benzoyl phenyl ureas (e.g. diflubenzuron), macrocyclic lactones (e.g. ivermectin), and neonicotinoids (e.g. imidacloprid).

An exception to this was the development of cyromazine and dicyclanil by Ciba Geigy (and subsequently Novartis) in the 1970s and 1980s. Cyromazine was developed as a larvicide for on-animal use to control the sheep blowfly, as a feed-through component of poultry rations for control of nuisance flies in poultry sheds, and for environmental (off-animal) treatment of nuisance fly breeding sites, during the 1970s. Its spectrum of activity was limited to fly larvae only. The early screening stages in the development of the compound showed that it was not effective against ticks, mites or adult insects of any type. The fact that work on cyromazine, and subsequently on dicyclanil, proceeded at Ciba Geigy despite this limited spectrum of activity seems to be due to a number factors that existed at the time, as described in an article by one of the people who worked on the compounds (Junquera 2017). These factors are described below, along with descriptions of why they no longer exist in the sheep blowfly insecticide market:

i) 1970s / 80s: the high wool price at the time meant that the insecticide use within the sheep industry was an attractive market in Australia and New Zealand for an animal health company.
Today: reduced value of the sheep industry and lower sheep numbers. There are now less than 50% of peak sheep numbers, alongside changes in the composition of the sheep flock, with less Merinos and many more meat breeds that are generally less susceptible to strike. Hence the flystrike chemical control market is much less attractive for companies relative to other livestock parasites (cattle tick, gastrointestinal worms), and even more so with regard to the market for companion animal parasite control products.

ii) 1970s / 80s: there was a market advantage in the fact that cyromazine represented an early example of an insecticide that showed low mammalian toxicity. The other chemical classes in use as insecticides at the time all showed significant toxicity to mammals: organochlorines, organophosphates, carbamates and amidines. In particular, there was some public pressure in the UK to reduce the use of organophosphate compounds
Today: this perceived strength of cyromazine no longer exists as most insecticides developed since that time are specific for insects, with many targeting ion channels that only exist in insects, eg, neonicotinoids. Hence, the novelty of cyromazine's insect- specificity in the 1970s no longer applies.

iii) 1970s / 80s: Novartis at that time, along with other animal health companies, had a focus on control of livestock parasites.
Today: the main focus of animal health companies has shifted to the companion animal market.

Hence, while cyromazine and dicyclanil were developed as insecticides despite having only larvicidal activity against flies, with markets in just the sheep and poultry industries, and for environmental control of nuisance flies, this would most-likely no longer occur at any animal health company that is involved in the discovery and marketing of new insecticides.

4.1.3 The current position of sheep flystrike in animal health company priorities

Some of the leading animal health companies have very extensive chemical screening programs aiming to discover new drugs for parasite control. The priority parasites for these companies are:

- Companion animal market (cats and dogs)
  - Heartworm
  - Fleas
- Livestock market
  - Cattle tick
  - Gastrointestinal nematodes (the cattle market more important than sheep)
  - Horn fly, buffalo fly (cattle)

The sheep blowfly is seen as a very low priority by the major companies. This will limit the attractiveness for animal health companies to invest in any effort to develop new blowfly control chemicals.

Screening of drugs against the blowfly is done on a small scale (as a follow-up screen for compounds shown to be hits against the priority parasites) in at least one company. However, even in this company, it is seen as a low priority compared to the other parasites.

Despite this position of secondary importance, it remains possible that new blowfly control products will be developed as the companies try to gain further markets once any new drug has established itself in the priority pest markets. However, this will depend on the market size of the blowfly control area at the time, and will be driven partly by the number of products that are available for flystrike control at the time. The impact of the currently-emerging resistance to dicyclanil in field blowfly populations will be an important issue here.

4.1.4 The blowfly genome and drug discovery

The blowfly genome was sequenced as part of the i5k Arthropod Genome Project which aims to coordinate the sequencing and analysis of the genomes of 5,000 arthropod species (i5K Consortium, 2013). Transcriptomes from various blowfly life stages were also generated as part of the project. The blowfly genome work was partly funded by Australian Wool Innovation, and led by the University of Melbourne, with the biological material (in-bred blowfly population, RNA / DNA) generated by labs at QAAFI and CSIRO. The genome and transcriptome were published by Anstead et al. (2015). Subsequently, a refined draft (v2) of the genome was generated by the University of Melbourne.

The genome contains many genes coding for proteins that are considered to be potential drug targets, including ion channels, G protein coupled receptors, GTPases, transcription factors, kinases and growth factor receptors (Anstead et al. 2015). These protein classes of potential targets are widely studied as potential drug targets across many insect Orders in research institutions and pharmaceutical companies worldwide. The genome therefore illustrates that many examples of these gene families also exist in the sheep blowfly.

The question arises as to the use of the blowfly genome for the development of new insecticides for blowfly control. As described above, animal health companies need to be the drivers of any insecticide discovery efforts as they will be ultimately responsible for bearing the very considerable costs associated with bringing any new insecticides to the market. From a commercial company perspective, the pathway to developing new insecticides needs to focus on the animal health priority

pests, as well as the Agrochemical company priority insect pests of broadacre crops, with the possibility of a subsequent development of new compounds for blowfly control once they have established their position in these priority markets. Hence, drug discovery efforts will probably not be led by investigations focusing on blowfly genes.

However, the genome may still be able to play a role in the initial stages of the drug discovery process. For example, the highlighting of a specific drug target in blowflies, and some validation of its worth as a drug target (for example, potency of prototype drugs against blowfly larvae), accompanied by evidence that the target is also present in a priority crop or companion animal pest, and that the prototype drugs are active against this priority pest, may be a useful means to attract the attention of the companies. In this way, work based on drug targets identified in the blowfly genome could be used as a first indication of the worth of an insecticide target, with subsequent drug development work focusing on a company priority pest. The eventual outcome, in terms of benefit for the sheep industry in the control of flystrike, may be the development of a new flystrike chemical that can, as described above, be a secondary outcome of a larger drug development exercise focused on another insect or arachnid species. That is, the blowfly genome may be useful for initiating a drug discovery effort, but it is unlikely to be the focus.

Anstead et al. (2015) described the presence of a large number of genes in the genome of the sheep blowfly that had not been identified in any genome at that time. It has been suggested that the presence of a large number of genes specific to the sheep blowfly could be exploited to develop blowfly-specific insecticides. However, given the focus of animal health companies on their priority pests, as described above, such blowfly-specific genes (and hence, blowfly-specific drugs) are unlikely to be of interest to animal health companies, and are therefore unlikely to be exploited for development of insecticides for flystrike control.

An illustration of both the use of the blowfly genome, and the limitations of focusing solely on the sheep blowfly as a model organism for drug discovery exercises, is provided by some recent work in this area at CSIRO and the University of Queensland (Kotze et al. 2015; Bagnall et al. 2017; Kotze and Fairlie, 2020). These studies initially identified genes coding for histone deacetylase enzymes in the blowfly genome. These enzymes are widely regarded as potential targets for the control of a number of parasitic diseases in humans (reviewed by Andrews et al. 2012). Inhibitors of these enzymes showed potent activity against blowfly larvae in *in vitro* assays (Kotze et al. 2015; Bagnall et al. 2017) and in larval implant sites on sheep (Kotze and Fairlie, 2020), at levels comparable to cyromazine. These enzymes occur widely across all insects, and hence did not represent blowfly-specific targets, however the research work focused solely on activity against this insect, and hence the worth of these enzymes as drug targets in other pests of animals has not yet been established. Hence, until activity against pests of livestock beyond just the sheep blowfly is demonstrated, the potential engagement of animal health companies in this area of insecticide discovery remains uncertain. In addition, other aspects of drug development, such as stability, mammalian safety and regulatory environment, remain to be determined.

4.1.5 Preserving the effectiveness of current flystrike control chemicals

Given the crucial role that insecticides play in flystrike control, and the time it may take for alternative control measures to have a significant impact in reducing the reliance on chemicals (as described elsewhere in this report), it is important to preserve the usefulness of the current set of flystrike chemicals for as long as possible. This requires efforts to ensure that insecticide resistance does not reduce their effectiveness to such an extent that protection periods are significantly

reduced, or even a complete loss of efficacy occurs (as happened for the benzoyl phenyl urea class of compounds in the mid 2000s).

As described in section 4.1.1, Levot (2014b), Sales (2020) and Sales et al. (2020) have clearly shown that resistance measured using *in vitro* assays is increasing in field-collected blowfly strains, with these studies also illustrating the potential impact that cyromazine and dicyclanil resistance could have on flystrike protection periods. The report by Sales et al. (2020) also suggested a reduced period of protection for an ivermectin-based product in their laboratory-pressured dicyclanil-resistant flies. Resistance to ivermectin and imidacloprid has been reported in other insect and arachnid pests, and hence may be expected to emerge in the sheep blowfly. The sheep industry would not want to be in a situation in which resistance was impacting significantly on the protection periods of flystrike control products containing all of the currently-available chemical classes given the difficulties and time periods associated with the development of any new chemical class for flystrike control.

Preserving the usefulness of the current chemical for as long as possible is therefore of vital importance to the industry. Methods to reduce the rate at which resistance emerges, and to minimise its impact once it starts to emerge, are well-known from the many plant- and animal-based industries that rely, at least in part, on the use of insecticides for pest control. The principal method is the use of drug rotations to ensure that selection pressure is not imposed on the pest population by repeated use of chemicals from the same chemical class. Secondly, it is well recognised, although, not widely applied in practice, that effective resistance management should utilise diagnostic tests to detect resistance and hence allow drug-use decisions to be made based on knowledge of what resistances exist in the target pest population (Van Leeuwen et al. 2020). Reasons for the lack of widespread adoption of such diagnostics include labour associated with sampling and testing, cost of labour in the diagnostic lab, test-procedure costs, lack of molecular markers for resistance, the time taken for the tests to be performed, and uncertainty about the practical implications of the resistance status diagnosis.

For the Australian sheep industry, both of these resistance management issues are currently being addressed to some extent:

- The FlyBoss web site provides advice to graziers on the use of rotation strategies for chemical use, and gives information on the flystrike control products available to graziers, and the chemical classes contained within each product. This should allow for the implementation of drug rotations among the different chemical classes. The web site also provides information on the use of the various insecticide products for complementary flystrike and lice treatments.
- The NSW DPI work on drug sensitivity in fly populations submitted by graziers in regions across Australia (led by Narelle Sales) provides valuable drug resistance updates. Information on susceptibility to each of the major chemical classes is provided to graziers who have submitted samples, and an overall picture of resistance across Australia is provided by this project team. However, the work does have shortcoming in terms of its limited breadth of coverage of the sheep industry, and the time taken for the laboratory test to be performed and information to be provided to the grazier (6-9 weeks). This time period is simply a fly biology issue as the laboratory needs to breed the flies for two generations to establish a large enough fly population to allow for the testing procedure to be performed. Despite this, the resistance testing at NSW DPI provides very valuable information to the sheep industry. We suggest that the resistance monitoring service offered by NSW DPI be supported.

Increased scale of this service may be warranted, with some level of industry-wide coordination.

The time taken to receive information on resistance using the current NSW DPI test (6-9 weeks) is an important issue. The time period between sample submission and the test result being communicated to the producer means that the information on resistance status is most applicable to the choice of insecticide class for the next season rather than the season in which the sampling is done. This is of value in being able to direct the choice of early season prophylactic treatment in the following season. In particular, detection of resistance to a dicyclanil-based product would provide the imperative to use an alternative chemical class as the early-season treatment the following year. Similarly, evidence of emerging resistance to ivermectin or imidacloprid could be used to direct chemical use to the alternative class in the following season.

However, we suggest that there is a need for a within-season resistance test for the blowfly. As described above, chemical choice (when considering resistance status as a key determinant) for an early season prophylactic treatment will rely on the resistance test conducted during the previous season. However, decisions on mid- or late- season second treatments, in seasons where they become necessary, would greatly benefit from a knowledge of the resistance status of the fly population at that time. While such second treatments should not utilise the same chemical class as the first (in following a drug rotation strategy), there still remains a choice between the two alternative classes currently available. A rapid resistance test could be used to identify the most suitable second treatment.

In addition, where the first treatment in a season is based on a threshold of fly activity, it would be of benefit to be able to test for resistance at the first sign of fly activity, and base the choice of chemical to apply to the mob on the resistance status test result. This would require a rapid turn-around of a few days to a week from fly sampling to the resistance status being defined.

A further aspect of preserving the useful life of insecticides for flystrike control lies in the exploration of whether it is best to use drug combinations (mixtures) or rotations, or some other approach, in resistance management. This subject is often controversial. Recently, the Resistance to Xenobiotics (REX) Consortium, a group of high profile international scientists working across disciplines in the area of resistance to pesticides and drugs (insecticides, herbicides, antibiotics, protease inhibitors etc), reviewed 16 published theoretical papers and found that the mixture strategy was superior to the rotation strategy in 14 of the 16 cases, with one case the opposite and another indeterminate (REX Consortium, 2013). More recently Sudo et al. (2017) compared insecticide management strategies across a range of different insect life histories and insecticide types. They found that the mixture strategy was optimal either when insecticide efficacy was incomplete or when some insects disperse between spatially-separated sub-populations before mating. The rotation strategy, which uses one insecticide in one pest generation and a different one in the next, did not differ from sequential usage in the time to resistance, except when dominance was low. It was the optimal strategy when insecticide efficacy was high and premating selection and dispersal occur. However, many empirical researchers still favour rotation in preference to the mixture/combination strategy (Insecticide Resistance Action Committee, 2012). Clearly the advantages of mixtures or rotations, or perhaps some other strategy or a mixture of both approaches (Newman and Busi 2016), are highly dependent on the particular pest and treatment strategies under consideration. One clear point with two component combinations however, is that to be most effective in delaying the development of resistance they should be employed before resistance is present to either of the mixture components.

No combinations products are available for sheep flystrike prevention in Australia although a combination product, containing cyromazine and ivermectin, is available for flystrike control in New Zealand (Cyrazin KO). However, the rationale behind the product appears to be for it to provide long-term flystrike control with cyromazine and ivermectin, alongside the knock-down of maggots in existing infections by ivermectin (that is, as a blowfly preventative and a dressing treatment), and to control existing body lice infestations (ivermectin), rather than as a means to delay resistance.

Combination products presently dominate the market for worm control in sheep, however, this alone should not be seen as an indication that the approach would be useful for flystrike control. There are many factors which are likely to determine the most efficient drug-use strategy for flystrike control, and need to be considered, for example the presence and nature of refugia, the mode of inheritance of the resistance to the combination components, the stage of the insect being targeted, cross-resistances to the component chemicals, prevalence of resistant individuals in field populations, and the relative persistence of the combination components. We could find no previous report of the use of modelling to inform the best strategy for managing resistance for sheep blowfly strike. Given the importance of maintenance of effective chemical controls for sheep flystrike and the likely slowing availability of new chemical actives for control in the future (discussed elsewhere in this document) we suggest that modelling to determine the likely optimal approach to drug-use patterns for resistance management in sheep blowflies should be a high priority.

4.1.6 Conclusions

The sheep industry currently relies on the use of insecticides to protect sheep from flystrike. Insecticide resistance has arisen in blowflies in the past, to the extent that several classes of insecticides became ineffective for flystrike control (organophosphates and benzoyl phenyl ureas). Recent work indicates that resistance to the widely-used cyromazine and dicyclanil-based products has emerged. While the exact prevalence of resistance remains unclear, results to date suggest that resistance to both dicyclanil and cyromazine may be relatively common. However, the precise impact of the levels of resistance currently seen in field populations on the flystrike protection periods of dicyclanil- and cyromazine-based products remains unclear. The sheep industry does have some alternative chemical classes (ivermectin-, spinosad- and imidacloprid-based products), thereby providing a potential means to slow the development of resistance through the use of drug rotations.

The sheep industry relies on animal health companies to bring new flystrike control insecticides to the market. However, these companies are focused on much larger markets for pest control products in livestock and companion animals. Development of blowfly control products is not a high priority. The sheep industry therefore will rely on new insecticides becoming available as a secondary outcome of company drug discovery programs focused on their priority pests. While the blowfly genome may assist in drug development through demonstrating that specific drug targets do indeed exist in the blowfly, it will most likely not be central to drug discovery programs compared to research focused directly on the company priority pests. Given the current focus in animal health companies on the discovery of parasiticides, particularly for the companion animal market, it is likely that new insecticide classes will be identified in the future, and that these may have application for flystrike control. However, the timeline for this is very unclear.

This highlights the need to maintain the effectiveness of the currently-available flystrike control chemicals for as long as possible; a number of strategies to consider: promotion of FlyBoss information on drug rotation strategies, investigating the merits of combination drug products for

resistance management, awareness of the current drug resistance status of field blowfly populations, and drug-use management guided by the use of rapid diagnostics to detect insecticide resistance.

4.2 Repellents

4.2.1 Introduction

There has been some interest in the use of repellents for flystrike control. Such repellents would act to prevent female flies from depositing eggs onto sheep, rather than acting as insecticides to kill blowfly larvae. There are a number of advantages in the use of repellents compared to insecticides; for example, repellents are generally much less toxic than insecticides, and hence the issues associated with environmental contamination are not as severe. For the purposes of this section of the present report, we consider two general types of repellents (James et al. 1985):

1) Vapour-based: acting through exposure of adult blowflies to chemical vapours, deterring them from landing on the sheep.

2) Contact-based: acting to deter the fly from laying eggs after the fly has landed on the sheep and made contact with the chemical agent in the wool of the animal.

4.2.2 Vapour-based repellents

The use of volatile compounds as insect repellents has been studied for many years (reviewed by Isman 2006, Nerio et al. 2010, Showler 2017). The most commonly used repellent is DEET (N, N-Diethyl-meta-toluamide, or diethyltoluamide). This compound was developed for use by the US military as a repellent in the 1940s, and entered civilian use in the 1950s. Picaridin (a synthetic analogue of the natural plant product piperine) has also been widely used as an insect repellent since the 1980s, with less irritation associated with its use on human skin compared to DEET. Much of the work on insect repellents has focused on plant-derived essential oils. These consist of volatile mixtures of hydrocarbons with a diversity of functional groups, with their repellent activity largely due to the presence of monoterpenes and sesquiterpenes. The historical use of repellents against wool and wound myiasis flies and nose-bot flies in sheep was reviewed by James (1985).

The volatile nature of vapour-based repellents means that they evaporate quickly. Thus, they have limited residual action, with the repellency generally lasting only hours often, or up to 1 -2 days in some cases (Baldacchino et al. 2013, Showler 2017, Zhu et al. 2014). Hence, the only viable option for the use of such volatiles against the sheep blowfly would be to use a slow release formulation in order to provide a prolonged period of protection.

An exception to this short-term effect was observed by Callander and James (2012) in laboratory-based experiments measuring the ability of tea tree oil (from *Melaleuca alternifolia*) to deter blowflies from landing on treated wool, and to supress the oviposition of those flies that did land on the treated wool. Earlier experiments on the mosquito repellency of tea tree oil applied to human forearms had shown that the effect lasted up to 2 hrs only (Maguranyi et al. 2009), in agreement with the short-term nature of repellency by essential oils in general. However, Callander and James (2012) found that a formulation, consisting of a stable emulsion of tea tree oil in water, applied to wool samples in laboratory tests could deter fly landing and oviposition for 44 days after treatment of the wool (the experiment was terminated at 44 days). This represented a significant level of persistence, not normally reported for essential oils in repellency tests with other Dipteran insects, and may be a result of extended release of vapours from tea tree oil dissolved in the abundant lipid coating in the dense covering of wool fibres in the sheep fleece. Importantly though, as noted by Callander and James (2012), the treated wool in these experiments had not been "subject to environmental effects such as rainfall, high temperatures and photo-degradation which would

normally be expected to reduce the protective period'. Experiments to look more closely at stability of tea tree oil in fleece under field conditions may be warranted, however, it is likely that persistency of the oil will need to be extended using a slow release matrix if it is to have any commercial potential for fly control. Yim et al. (2016) reported on the use of β-cyclodextrin inclusion complexes to extend the period of repellency of tea tree oil against cattle tick larvae in *in vitro* assays, suggesting that possibilities exist for extension of their period of action against the blowfly through further work on controlled release formulations.

A great deal of work has been undertaken to develop slow release formulations for volatile agents that can be used to repel mosquitos in order to prevent transmission of mosquito-borne disease in humans. Many different controlled-release systems have been described: polymer micro /nanocapsules, nanoemulsions, liposomes, nanostructured hydrogels, and cyclodextrins (Tavares et al. 2018). However, most of these still only provide protection for 1-2 days. This time period is satisfactory for the purpose of mosquito repellency as they can be readily reapplied by humans on a daily basis, but clearly unsuitable for protection of sheep against flystrike.

Mapossa et al. (2019), recently described a formulation providing protection against mosquito bites for a much longer time period. They described the incorporation of volatile mosquito repellents into microporous polyolefin strands designed to be worn as an ankle bracelet by humans for protection against mosquitos. The bracelets provided effective protection against mosquito bites in cage studies after aging at 50^{o}C for 12 weeks. Importantly though, the bracelet was worn at the site that was presented to the mosquitos in the 'foot-in-cage' protection trials, namely, the ankle. By analogy, such devices would need to be deployed near the breech of sheep in order to protect against breech strike. Also, the bracelet itself consisted of a long strand that was wrapped repeatedly around the foot of human subjects, and hence was not practical in this form for use at the sheep breech.

Short-term repellency with vapour-based repellents plays a small role in current blowfly control through their use in strike dressing products. In this instance, the short term nature of their action is sufficient to deter blowflies from ovipositing at the site while a wound heals, alongside the direct insecticidal action of the organophosphate compounds that are also contained in these strike dressing products. Example include: WSD Aerosol Sheep Dressing®, containing dibutyl phthalate alongside the organophosphate compound chlorfenvinphos, and Defiance® S Aerosol Insecticidal Flystrike, Mules and Wound Dressing containing eucalyptus oil, cresylic acid and naphthalene alongside chlorfenvinphos.

4.2.3 Contact-based repellents

4.2.3.1 GH-74

Work conducted by George Holan at CSIRO the 1960s aimed to modify the DDT molecule in order to reduce its mammalian toxicity and increase its biodegradability, while retaining insecticidal activity (Holan 1971a, b). One of the compounds that showed significant potency in *in vitro* assays with the housefly, *Musca domestica*, was 1,1-bis(4-ethoxyphenyl)-2-nitropropane (abbreviated as ENP, and also referred to as GH74).

Barton-Browne and Gerwen (1982) reported that GH74 was a potent oviposition deterrent for the sheep blowfly. Sheep were treated by jetting with GH74, and sections of fleece were removed at various times over a 40 week period, and exposed to gravid female flies in cages. The total number of whole egg masses deposited over 13 experiments at various time points up to 40 weeks after jetting was 1392 for control fleece, compared to 35 on treated fleece. In the period up to 26 weeks

after jetting, the total egg masses laid on control fleece samples was 1102, compared to 21 on treated fleece. Oviposition on live sheep following jetting with GH74 was also measured: over time periods ranging up to 41 weeks, 378 full egg masses were laid on control sheep compared to 15 on treated sheep. These results clearly indicated that the compound was a potent and long-lasting oviposition suppressant.

However, the compound was not as effective around the breech of scouring mulesed sheep. In one experiment, the number of egg mases on sheep (treated by jetting) at 3 weeks was less than controls, however, there was still a significant degree of oviposition on the treated animals. In a second trial, there was significant oviposition on treated sheep 7 weeks after jetting. Importantly, the placement of egg masses around the breech was different for control and treated animals: egg masses on control sheep "were widely distributed within the area of faeces-soiled fleece" on these scouring animals, whereas they were only present at the edges of the wool around the breech on treated animals. This suggests that the flies on the treated animals had most-likely stood on the wool-free area of the breech to lay their eggs. This positioning would have allowed the flies to avoid direct contact with the GH74 present on the wool fibres.

Hence, while the compound seemed to show significant potential as an oviposition deterrent to prevent body strike, its effectiveness in preventing breech strike in scouring mulesed sheep was limited. This limitation may however be less of an issue today as more producers switch to a non-mulesing operation, and hence are providing less bare skin for adult flies to position themselves on to oviposit in order to avoid contact with any applied chemical. On the other hand, even in non-mulesed sheep, female flies may still be able to avoid contact with a chemical by positioning their body on faecal material of a scouring sheep while they oviposit, and hence still initiate breech strike. A further consideration is that selection of animals based in breech cover in breeding programs aiming to reduce flystrike will increase the area of natural bare skin around the perineum and breech area, and hence provide surfaces for ovipositing flies to avoid exposure to chemicals acting as described for GH74.

A subsequent study by van Gerwen and Barton Browne (1983) focused just on body strike, and again showed significant deterrence of oviposition: 1) very low numbers of eggs laid on sheep jetted with GH74 compared to controls for a periods up to 28 weeks after treatment, 2) again, very little oviposition on sheep tip-sprayed with GH74 compared to controls for periods of up to 18 weeks after treatment.

The results of these two studies show that treatment of sheep with the compound GH74 provided a very significant level of protection against oviposition by blowflies, associated with body strike, for prolonged periods.

No detailed information is available on the reasons behind the failure of this compound to progress to commercial development, other than the following comments from Colin Ward (ex-CSIRO), indicating that costs of synthesis at the time were the main obstacle. These comments also indicate that the compound had potential use well beyond the sheep blowfly:

"One compound that showed early promise, called GH74, had a slightly modified base and did not contain chlorine. With activity equivalent to DDT, low toxicity, and a structure that would not pose the residual problems that were emerging with DDT, it appeared set to enter commercial development by Wellcome Co. in the UK who expended £1.2 million in its development worldwide. Unfortunately, the sudden onset of the 1974 energy crisis pushed the cost of the petrochemicals necessary for its synthesis to uneconomic levels and development had to be aborted." (Ward 2011a)

"This compound progressed to chronic animal toxicology tests and into worldwide field trials against a large range of animal and human insect pests. GH74 was accepted by the World Health Organisation (WHO) for large-scale trials in Africa for the control of *Simulium damnosum* (blackfly) the carrier of onchocerciasis (river blindness) and the tse-tse fly (trypanosomiasis, sleeping sickness carrier). Trials were carried out by WHO at Buake (Ivory Coast) and Tano River District (South Kenya), respectively. The trials were successful but the development of GH74 was abandoned on the advent of 1974 world energy crisis (raw materials cost)." (Ward 2011b).

4.2.3.2 Synthetic pyrethroids (SPs)

While SPs are used widely as insecticides for killing insect pests, most SP insecticides also inhibit oviposition (Ruscoe 1977). This is likely due to their rapid action on sensory neurons, including those that control the fly's ovipositor. Studies on the effects of these insecticides on oviposition by adult female sheep blowflies led to their development as oviposition suppressants. The first product registered was Sectar®, containing cypermethrin and diazinon (contents described by the Australian Agricultural and Veterinary Chemicals Council, 1990). This product was described as a "sheep blowfly strike suppressant and long wooled sheep lice treatment". The cypermethrin acted as an oviposition suppressant while the diazinon acted as a larvicide, with the cypermethrin also providing control of cypermethrin-susceptible lice. The product was described as being able to supress both body and breech strike. Today there remains only one SP-based blowfly control product: Vanquish®, containing alpha-cypermethrin, with a claim for protection against body strike only, for up to 10 weeks.

SPs also act against the larval stages of the blowfly (Sales et al. 1996). At the concentrations of the products applied to sheep, the SPs would act as both oviposition suppressants and larvicides (Orton et al. 1992, Sales et al. 1996).

The widespread use of SPs against lice before their introduction for fly control initiated an investigation into whether this previous use for lice control may have led to resistance developing in blowflies, with the possibility that this may decrease their sensitivity to oviposition suppression. Sales et al. (1996) examined larvicidal and oviposition responses in a number of field-collected blowfly populations (n > 100) and found that the two responses were not correlated. Further, they reported that while there was some evidence for reduced sensitivity to the larvicidal effects in the field strains compared to a reference drug-susceptible strain, the field strains behaved similarly to the reference strain in terms of oviposition responses. This indicated that while the previous exposure had likely led to decreased sensitivity in the blowfly larval stages, the potency of the SPs for oviposition suppression remained unaffected. Whether this situation still exists today is unknown.

There are clear advantages in using products that have a distinct modes of action against blowflies in order to delay resistance development. The oviposition suppression activity of SPs has a further benefit in targeting a different life stage of the blowfly. We suggest that awareness of the role of SPs in blowfly control as an alternative, to use in rotation with other chemical classes, be maintained. The inability of the presently-available product to control breech strike is however a significant disincentive, and may warrant consideration of development of a combination product containing an SP and another active. Such developments in the use of SP-based products will be determined by animal health companies.

An important consideration in the continued use of SPs is the residue limits imposed on wool products in some markets, particularly the European Union. As noted in LiceBoss, 'SPs must not be used, even off-shears' to satisfy European Eco-label wool residue requirements.

4.2.4 Conclusions

Vapour-based repellents act for only very short periods due to their volatile nature. This action is however useful for current strike-dressing products as it provides some repellency for the short period of time while wounds heal. The use of vapour-based repellents to provide long-term protection against flystrike will depend on advances in controlled-release technologies. Such advances may come from work currently underway to provide slow release of insect repellents preventing transmission of important vector-borne diseases such as malaria.

Long-term protection against flystrike was provided by the contact-based repellent GH74 in experiments conducted in the 1980s. It seems that the development of this compound did not proceed due to costs associated with the chemical synthesis pathway. SPs play a minor role in flystrike protection currently, with only one SP-based product currently on the market. Two positive aspect of their use are, firstly, that they provide some control using a different class of chemical to the widely -used products on the market (and therefore may not be affected by resistances that arise to these other chemicals), and, secondly, in acting as a repellent against adult blowflies they are targeting a different life stage compared to the other products which act as larvicides only. However, a negative aspect of their use is the high concentration of chemical used to provide practical periods of protection, alongside the residue limits imposed by some markets, particularly the EU.

4.3 Biological Control

4.3.1 Categories of biological control agents / biopesticides

The use of naturally occurring biological pathogens, such as parasitic wasps or insect predators, nematodes, bacteria, fungi and viruses has long been a focus in the search for non-chemical approaches to the management of livestock parasites (Sandeman et al. 2014, Leathwick et al. 2019).

Biological control can be considered in three main categories:

- classical or inoculative biocontrol
- inundative biocontrol
- the use of plant, arthropod or other biologically derived toxins.

In classical or inoculative biocontrol, parasites, predators or pathogens are released into the pest population and are expected to persist, multiply and spread to bring about ongoing suppression of the target pest. A subset of this method is augmentative biocontrol where extra numbers of an already present organism are added to the system to increase numbers of the biocontrol organism. An example of this is the release of parasitoid wasps to control nuisance flies in cattle feedlots or poultry facilities where extra numbers of a wasp already present are released to aid early season build up or increase wasp numbers and improve control of flies.

The second major category is inundative bio control (microbial pesticides) where large numbers of a living organism are applied to flood the pest populations as a biological pesticide. In this case it is not expected that the agent will persist in the environment to give ongoing control, but rather that it controls or eradicates the pest and then returns to low levels or dies out completely. A number of different types of pathogens have been considered for innundative control of sheep blowflies including fungi (Cooper and Pinnock 1983, Leemon and Jonsson, 2012), entomopathogenic nematodes (Bedding, 1983), bacteria (Lyness et al. 1994; Gough et al. 2005) and viruses (Thomson and Bushell 1983).

The use of plant derived compounds, arthropod or other animal derived toxins, or ribonucleic acid molecules (gene-silencing technologies) can also be considered biocontrol, and for the purposes of this review has been included here in section 4.3.7.

4.3.2 Parasites and predators

In the early 1900s prior to the development of chemicals for fly strike control significant research was conducted into the possibility of classical biological control (Anon 1933). At this time it was believed that the majority of *L. cuprina* bred in carcases, although this is now known not to be the case. Between 1900 and 1920, large numbers of a naturally-occurring pupal parasite *Mormoniella (Nasonia) vitripennis* were bred and distributed (Froggatt 1918). It was concluded as early as 1921 that they were unlikely to be successful as they bred more slowly than *L. cuprina* and lost efficiency by superparasitism (more than one wasp laying its egg in the same pupae or larvae) and by ovipositing in puparia too advanced for emergence of the adult flies to be prevented (Johnston and Tiegs 1943). In addition, Fuller (1934) found that this parasite preferentially oviposited in the larvae of secondary flies which compete with *L. cuprina* in carcases and thus probably actually favoured survival of *Lucilia*. *Alysia manducator*, a parasite of *Lucilia sericata* in Europe was introduced in the 1920's. However, this parasite prefers to oviposit on carrion in the shade, and prefers cool moist conditions, and failed to become established. Predatory beetles were also studied but it was found

that they mainly attacked larvae which left the carcase early and which would have died anyway (Fuller et al. 1934).

In New Zealand, investigation of parasitism in larvae collected from flystrikes on sheep identified three species of parasites of *Lucilia* spp. larvae, *Tachinaephagus zealandicus*, *A.manducator* and *Aphaereta aotea* (Bishop et al. 1996). However, the overall parasitism rate was very low (1.1%) and unlikely to contribute in any significant measure to the regulation of blowfly populations.

4.3.3 Entomopathogenic fungi

In the early 1980s a spore-forming unicellular parasite, *Octosporea muscaedomestica,* at that time considered a protozoan but now known to be a fungus, was imported from the United States for potential release as an inoculative biocontrol for *L. cuprina* (Cooper et al. 1983). *L cuprina* was highly susceptible to this pathogen, but it took a number of days for infection to develop and infected flies were able to successfully oviposit before infection could impair fertility (Smallridge et al. 1995). In addition, maintaining spread of this pathogen in sheep blowfly populations would be problematic. Infection is spread mainly at feeding sites through spores in faeces passed by infected adult flies. As *L cuprina* have few common feeding sites, can obtain protein for egg laying from a wide range of sources, and can persist in the field at low densities for most of the year, achieving sufficient transmission to significantly reduce fly numbers was considered likely to be difficult and this approach was not pursued.

A number of species of entomopathogenic fungi have been tested for their effect against myiasis flies in Australia (Leemon and Jonsson, 2012), New Zealand (Wright et al. 2009) and in the United Kingdom (Wright et al. 2004). In Australia 24 isolates of *Metarhizium anisopliae* and eight of *Beauveria bassiana* isolated from soils and infected insects in Queensland were selected on the basis of characters suggesting suitability for mass production, and then screened against adult and larval *L. cuprina* (Leemon et al. 2012). Adult flies exposed to conidia in their food died faster than those challenged topically with three strains giving 100% mortality within 5 days.

Wright et al. (2004) considered *M. anisopliae* for use as an inoculative biocontrol in Britain. They concluded that with appropriate lure and kill systems sufficient levels of infection could be introduced into *L. sericata* populations to induce the 20%-30% daily mortality that modelling suggested was required to bring about a reduction in adult *L. sericata* populations. However, under Australian conditions, given the low dispersal of *L. cuprina* , the relatively limited distances of attraction apparently possible with current *Lucilia* attractants, and the high density of traps required to bring about reductions in strike incidence using a trap and kill approach (see section 4.9) (Mackerras 1936, Ward and Farrell 2003) it is questionable whether *M. anisoplae* used with a lure and kill system could bring about sufficient reduction of *L. cuprina* populations to provide significant effects against flystrike.

Wright et al. (2009) in New Zealand screened dead larvae and pupae of closely related *L. sericata* for pathogens. Although 38 bacterial and fungal isolates were isolated, in most cases infected flies were able to successfully complete their life cycle and most of these species were considered unlikely to be of use in biocontrol. However, two strains of an entomopathogenic fungus *Tolypocladium cylindrosporum* strongly affected development of *Lucilia* spp. with only 0-20% of infected larvae completing development to adult flies. These authors suggest that this fungus could potentially be used to control the soil stages of sheep blowflies, possibly targeting the overwintering stages of the larvae or could perhaps be applied directly to sheep to prevent or treat strikes.

In tests of different *M. anisopliae strains* against *L cuprina* larvae, three isolates added to the larval medium killed 100% of first instar larvae and 12 isolates added to the pupation medium killed 100% of third instar larvae (D. Leemon pers.com.). Neither these isolates or *T. cylindrosporum* have yet been tested on sheep. However Wright et al. (2009) suggested that the fleece may not be a favourable habitat for fungal growth and Heath et al. (2004) suggested that protection times provided by these biocontrol organisms may be limited by wool growth which carries them away from the skin where the larvae feed.

More recently, Muniz et al. 2020 showed that the entomopathogenic fungi *B. bassiana*, *B. pseudobassiana* and *A. muscarius*, were toxic to adult *L. sericata* and that toxicity was enhanced when the fungi were formulated in electrically charged carnuba wax. However in this case the LT50's (time to kill 50% of the flies) were still 6.9, 8.6, and 13.8 days respectively, suggesting that a significant proportion of flies may still survive long enough to oviposit. The difficulties for use of methods that target adult flies, with either chemical or biological treatments, in providing practical periods of control against sheep blowflies in Australia are addressed elsewhere in this review (section 4.9 Trapping). Under Australian conditions, use of fungal biopesticides by application to sheep, to target first instar larvae, may have greater potential for practical control than inoculative approaches.

4.3.4 Entomopathogenic nematodes

A further group of organisms that have been suggested to be potentially of benefit for controlling *L. cuprina* are entomopathogenic nematodes (ENs). ENs kill their host through release of mutualist bacteria which then proliferate in the dead insect, suppressing the growth of competing bacterial species and providing food for the succeeding generations of nematodes. When the nutrition provided by the dead insect is exhausted, juvenile infective nematodes leave the insect cadaver to search for a new food source.

An attraction of ENs as biopesticides is that they are motile and can actively seek out their hosts to infect and kill them. First instar *L. cuprina* larvae become infected by entrance through the mouth, spiracles or anus, or in the case of one species of EN, by direct penetration through the cuticle (Bedding and Molyneux, 1982). *L. cuprina* pre-pupae in the soil were shown to be quite susceptible, possibly due to their relative inactivity (Bedding, 1983). As the majority of the strains of ENs are most active at relatively low temperatures, it has been suggested that ENs could be used to increase mortality in the overwintering soil stages of *L. cuprina* (Bedding, 1983). Little infection of pupae was seen in the Australian studies, but in a laboratory study with *L. sericata* and a different nematode strain, application of high concentrations of nematodes to soil gave up to 70% infection of pupae (Mahmoud et al. 2007).

The potential of these organisms to infect prepupal larvae is attractive if locations where prepupae and pupae concentrate in the soil can be identified. As most larvae leave sheep to pupate during the night when the sheep are in sheep camps or near waters it might be hypothesised that introduction of ENs in targeted areas, such as sheep camps, could increase mortality in *Lucilia* populations. Mortality induced by ENs could be particularly important during overwintering when large numbers of prepupae and pupae die anyway, and further reduction of the overwintering populations could have significant effects on flystrike incidence in the next season (McKenzie and Anderson 1990). However, the preference of most ENs for a humid microenvironment to achieve high levels of infection and persist through prolonged periods when few host larvae are present may preclude the use of ENS as an inoculative control against the soil stages of *L. cuprina* in many Australian situations. In addition, much better knowledge of the spatial and temporal ecology of the soil phases

of *L. cuprina* will be required to assess whether sufficient mortality of larvae could be induced to significantly affect sheep blowfly numbers.

When tested to see if ENs could be effective for control of strikes on sheep the nematodes survived in the fleece for several days, but there was only a low larval infection rate (Bedding, 1983), probably because of the relatively high temperatures at the skin surface of struck sheep. There are also a number of practical concerns associated with treating live sheep with nematodes, that would probably render this approach impractical, for example the persistence of dead and decomposing larvae in the fleece.

4.3.5 Bacteria

4.3.5.1 *Bacillus thuringiensis*:

Probably the most-studied biological agent for control of the sheep blowfly has been the soil bacterium *Bacillus thuringiensis* (*Bt*). The primary habitat of *Bt* is in the soil but it is widespread in the environment with different strains varying widely in their toxic activity. *Bt* can produce a number of classes of toxins that can be involved in conferring insecticidal effect including δ-endotoxins, which are the proteins of most interest, vegetative insecticidal proteins (Vips) associated with the vegetative growth phase of *Bt* , and β-exotoxins (thuringiensin), which inhibit RNA polymerase and are highly toxic to most forms of life, including *L. cuprina* larvae. Strains of *Bt* that produce β-exotoxins are not permitted for use in commercial *Bt* products because of their high mammalian toxicity.

The main insecticidal proteins of interest produced by *Bt*, and which have been widely used in pest control, are the δ-endotoxins. These proteins are toxic to insects but have no mammalian toxicity (Siegel 2001). Following ingestion by the insect, the endotoxins are activated by proteolysis in the insect gut, and the activated toxin then induces the formation of pores in the midgut epithelial cell membrane, leading to increased membrane permeability, gut paralysis, cessation of feeding, and eventually death of the insect. Different strains of the bacterium produce different endotoxin proteins that show specificity at the level of insect Order, that is, Lepidoptera (caterpillars) vs Coleoptera (beetles) vs Diptera (flies) vs Hymenoptera (wasps) etc. As a group, the endotoxins show activity against a wide variety of insects, including important pests of broadacre crops, horticultural crops and livestock, as well as nuisance flies and some Dipteran vectors of human disease, and nematodes (Fietelson et al. 1992; Schnepf et al. 1998; Lacey et al. 2001). They are used widely as a spray for protection of crops against lepidopteran pests. In addition, a number of species of transgenic crop plants expressing one or more *Bt* endotoxin genes are now being grown commercially (for example, *Bt* cotton in Australia). There are more than 42 different classes of insecticidal proteins found in *Bt* with only some of them shown to be active against sheep blowflies. A number of field-isolated strains of *Bt* have been shown to produce toxins that are active against blowfly larvae in *in vitro* bioassays (Lyness et al. 1994; Heath et al. 2004; Gough et al. 2005; Kongsuwan et al. 2005).

A number of *in vivo* studies have also been reported:

1) Lyness et al. (1994); Sheep were treated by jetting with a *Bt* spore suspension, and protection from flystrike was assessed using a series of larval implants. In this study protection for 11 weeks was reported at the highest *Bt* concentration. The nature of the *Bt* toxins, that is whether the suspension contained β-exotoxins and / or δ-endotoxins, was not reported.

2) Heath et al. (2004); *Bt* extracts were applied to patches on sheep, and then the ability to protect from flystrike was assessed using larval implants over time. A separate large field trial involved the treatment of sheep with *Bt* solution along the midline and around the rump, and then exposure to natural flystrike. The *Bt* was able to protect sheep from experimentally- induced flystrike (implants) for up to 6 weeks. A time course study showed that protection from flystrike was not diminished by exposure of sheep to precipitation or sunlight. Rather, the loss of protection over time was considered to be most-likely due to movement of the toxin away from the skin as the wool grew. In the field trial, the *Bt*-treated animals showed 36 % fewer strikes than control animals, but the difference between control and *Bt*-treated animals was not statistically significant. In addition, *Escherichia coli* expressing Cry 1Ba protein applied to sheep did not protect against strike, even though it was toxic to larvae in laboratory assays.

   A separate component of this study examined whether a *Bt* strain could colonise the fleece and hence exert on-going larvicidal activity. However, it was found that vegetative forms of the Cry1Ba protein-producing strains did not establish on sheep, did not produce significant sporulation, and no protection against fly strike was achieved.

3) Gough et al. (2005); a small scale experiment using foam ring-enclosed larval implant sites on 5 sheep, showed that a relatively high concentration of *Bt* extract resulted in 100 % larval mortality. The persistence of protection was not assessed.

Despite long-term interest, no commercial *Bt* products have yet been registered for sheep ectoparasite control.

4.3.5.2 *Brevibacillus laterosporus:*

Another well known bacterial pathogen of insects, tested for effect against *L. cuprina* is *B. laterosporus*. Fourteen strains were tested in laboratory studies, and induced larval mortality varying between 29% and 54% (Pessanha et al. 2015) and few biological effects were seen in surviving stages, suggesting it is unlikely that this bacterium could be developed to provide useful levels of field control.

4.3.5.3 *Wolbachia:*

*Wolbachia i*s a genus of intracellular, maternally-transmitted bacteria that can infect a range of arthropod species and filarial nematodes. *Wolbachia* are somewhat different than the other biocontrols in that they are vertically transmitted from mother to offspring in the eggs and can spread through insect populations by manipulating host reproductive processes. This makes them less density dependent than horizontally transmitted organisms, although a certain initial release ratio is generally required for the establishment of *Wolbachia*. With recognition of the immense potential for use of *Wolbachia* in the control of insect pests and insect-vectored disease, interest in this organism has increased exponentially in recent years (Floate et al. 2006). The use of *Wolbachia* in novel control approaches is currently being studied in for a range of insect pests and human diseases (e.g. dengue fever transmitted by mosquitoes in northern Queensland) (McGraw and O'Neil 2013), but as yet has received little attention for the control of veterinary pests.

Wolbachia infection has many and varying effects depending on the host context. The major effects of potential relevance to sheep blowfly control are cytoplasmic incompatibility (CI) and effects on host fitness. *Wolbachia* infection can interfere with insect reproduction in a number of ways but one of the most common effects is cytoplasmic incompatibility (CI) whereby matings between infected males and non-infected females, or between males and females infected with different strains of

Wolbachia, produce infertile eggs. This means that infected females that mate with similarly infected males are fertile and pass on the infection to their progeny whereas uninfected females are rendered sterile and don't breed. In this way *Wolbachia* can spread itself through a population (Figure 1).

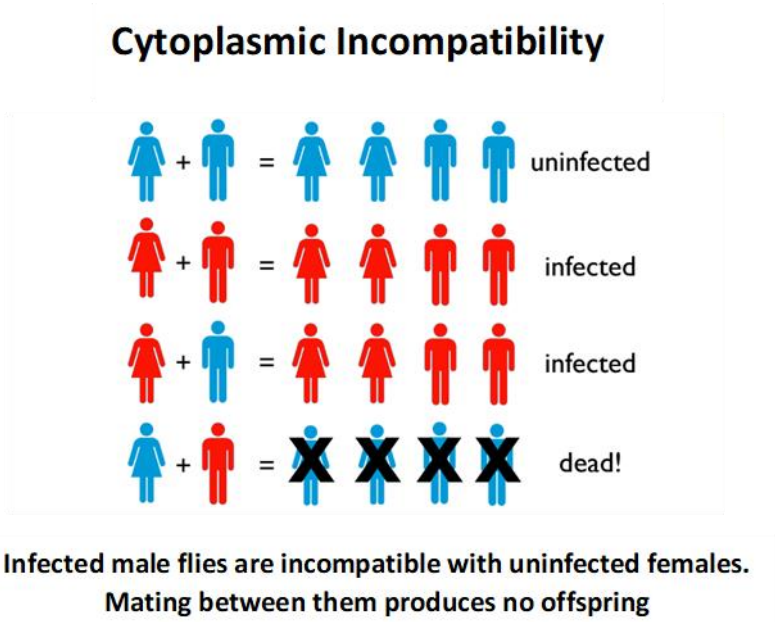

Figure 1: Schematic representation of Wolbachia-induced cytoplasmic incompatibility. Matings between infected males and non-infected females are not fertile and produce no fertile eggs. However, matings of both infected males and non-infected males with infected females are viable and produce fertile, infected eggs; adapted from Werren et al. (http://www.sas.rochester.edu/bio/labs/WerrenLab/WerrenLab-WolbachiaBiology.html)

Infection with *Wolbachia* has also been shown to induce a wide range of fitness effects in infected insects such as reducing insect lifespan, decreasing egg viability, reducing pupal emergence, and reducing mobility and feeding efficiency (McGraw and O'ONeil 2013). These effects can have significant impact on survival and reproduction in insect populations. Richie et al. (2015) proposed that similar effects in mosquitoes could be used to "crash" local populations during the overwintering phase. A similar approach whereby *Wolbachia* spread through sheep blowfly populations under the effects of CI during the blowfly season, and then causes compromised survival in the overwintering larvae, could also be contemplated with sheep blowflies.

CI can also be used for direct suppression of insect populations in a similar manner to the sterile insect technique (see section 4.11) in a method known as the Incompatible insect technique (IIT). The potential effectiveness of using *Wolbachia*-induced CI in this way was demonstrated as early as the 1960's when release of *Wolbachia*-infected male *Culex quinquefasciatus* mosquitoes led to local elimination of this species from areas in Burma (Laven 1967) and a similar approach with *A. aegypti* mosquitoes in northern Queensland is currently producing encouraging results (Pagendam et al. 2020).

Although few blowfly species have been tested for the presence of *Wolbachia* (Madhav et al. 2020), infection has been reported in the bird-infesting blowfly *Protocalliphora* (Baudry et al. 2003) and has more recently been detected in *L. cuprina* from laboratory colonies at the Department of Agriculture and Fisheries, Brisbane, and at the University of Melbourne in field collections from a number of sites around Australia (Perry pers com.). Directly targeting *L. cuprina* populations by an

area wide approach based on a biological method, rather relying on individual animal treatments would provide significant on-farm labour savings and would enhance the reputation of Australian wool and sheep meat as clean, safe and ethically produced. However, the biological effects of *Wolbachia* in *L cuprina* are yet to be characterised.

4.3.6 General comments on inoculative biocontrols for sheep blowfly

Biological controls are generally considered to be more environmentally friendly, less prone to residues and safer for the operator than traditional chemical insecticides, and have been suggested to be less prone to the development of resistance, although this requires confirmation. In addition, agricultural products produced in low-chemical and organic systems generally have more favourable consumer acceptance, sometimes accompanied by price premiums. Despite this, to date no natural biocontrols that appear to exert significant regulating influences on sheep blowfly populations have been identified and the population dynamics and life history of *L. cuprina* would seem to present significant difficulties for any classical biological control agent to exert meaningful levels of control.

A general issue for most types of inoculative control is that *L. cuprina* occurs at low population density at most times of the year and flystrike waves are episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites in populations is almost invariably density-dependent, and spread through *L. cuprina* populations is likely to be inefficient at most times of the year, particularly when numbers of *L. cuprina* are very low. In addition, there is generally a lag time between build up in pests and build up in their parasites or pathogens. It is likely that a flywave would build and be over before inoculative biocontrol agents could build to levels where they could exert any significant controlling influence. Inoculative control in most instances will be self-perpetuating and is unlikely to provide a return to commercial investors, and as such, the development of inoculative biocontrol approaches will generally need to be funded by industry organisations or government.

However, even though inoculative biocontrols are unlikely to be effective as a standalone method for flystrike control, they could play a significant role as part of an integrated approach. Most previous studies have focused on the adult flies and the on-sheep larval stages but there has been little investigation of the potential for targeting the pre-pupal and pupal stages in the soil. Heavy mortality occurs during the soil stages varying from 50%-75% (Foster et al. 1975) to more than 90% in the overwintering stages (Whitten et al. 1977) and reducing the numbers of flies that survive overwintering could have significant effects in the following fly season, as demonstrated by McKenzie and Anderson (1990). However there is little information available on the spatial distribution of larvae and pupae in the soil or of temporal and spatial the ecology of soil pathogens to assess whether targeting the soil stages could provide a significant effect on fly numbers, or more importantly, strike incidence.

The use of *Wolbachia*-based approaches is now under investigation for control of a wide range insect pests, and offers potential for use as a biocontrol with *L. cuprina*. Importantly, as it is vertically transmitted and can manipulate host reproduction to actively spread itself through pest populations from a relatively low starting level (Kreisner et al. 2013). *L. cuprina* is known to be a competent host for *Wolbachia*, and given the substantial benefits from an area wide approach to control, the potential use of *Wolbachia* warrants further investigation.

Most recent research towards a biological control agent for sheep blowflies has focussed on the potential for inundative approaches where bacteria, fungi, nematodes, or potentially entomopathogenic viruses (Thompson and Bushel, 1983) could be sprayed onto the fleece as

'biological pesticides' to prevent strikes on sheep. This approach could bring most of the advantages of biological controls, in terms of 'clean green and sustainable and consumer perception, and although registration is still required for bacterial fungal and virus based formulations (though not entomopathogenic nematodes or macroscopic parasites), the registration pathway is usually somewhat simpler and considerably cheaper. However, in many instances the practical competitiveness of biopesticides is limited by significantly shorter periods of protection in comparison with chemical pesticides. In addition, the production of live biopesticides is often much harder to scale up than the with chemical pesticides, they can be subject to variability in efficacy, generally have shorter shelf life, are more prone to breakdown under environmental influences and can have difficulties with quality control. Nevertheless bacterial, fungal, and viral nematode formulations are all currently under commercial production for other pest control usages, and although they currently represent a relatively small share of the pest control market, this share is expected to grow (Ravensberg 2011).

4.3.7 Plant extracts, arthropod venoms, ribonucleic acid molecules

Botanical pesticides, or other biologically-derived pesticidal compounds, are considered to be much more 'eco-friendly' than synthetic chemicals, as they are less stable in the environment and usually have a narrower spectrum of activity. Some of the important differences between synthetic drugs and biopesticides / biologicals are described here as they impact on the likelihood of the commercial development of the latter for pest control markets, including for control of the sheep blowfly:

<u>Disadvantages of biologicals</u>

- Synthetic drugs are produced by defined and predictable chemical processes, whereas the production and composition of biologicals can be variable.
- Synthetic chemicals generally have more simple structures than biologicals, which are usually very large, complex molecules or mixtures of molecules.
- The production process of chemical drugs is relatively well defined, which allows these drugs to be produced in uniform large quantities. Biologicals, however, have a complex production process that tends to yield small quantities.
- It can be difficult to scale up biologics from laboratory quantities used for early analysis and preclinical testing to larger-scale batches and maintain product purity and batch-to-batch equivalence.
- Synthetic chemicals are generally more stable than biologicals which are often extremely sensitive to physical conditions (temperature, shear forces, chemical phase, and light) and enzymatic action; this instability is particularly relevant when considering suitability for use in prophylactic treatments to protect sheep from flystrike.

<u>Advantages of biologicals</u>

- Many biological pesticides have a narrower spectrum of activity. They may target specific pests and exhibit limited non-target effects, and thus pose minimal adverse effects on humans and the environment.
- The lack of stability (described above as a disadvantage in terms of the longevity of toxicity against the target pest) is beneficial in terms of the rapid degradation in the environment, and therefore lower risk of environmental effects
- In some parts of the world, there are different regulatory requirements for synthetic chemicals compared to biologicals, with the latter generally having lower hurdles to regulatory approval than synthetic chemical products. Some of the most costly health, safety

and ecological trials that are required for chemicals (e.g. carcinogenicity and reproductive effects) are waived for biological chemicals or extracts. These differences in the nature of regulatory issues and positive perceptions of biologicals versus synthetic chemicals, are illustrated on the APVMA web site (accessed July 13, 2020) –

- 'The APVMA recognises that, due to their inherently lower risk, some biological products may be more desirable than some synthetic pesticide chemicals'.
- 'Plant oils that cannot be totally characterised are classed as biological products. Similarly, plant extracts where the level of purification is incomplete and the chemical composition of the active constituent cannot be fully characterised are classed as biological products.'
- 'Biological products are not always as efficacious as synthetic pesticides and it may be sometimes necessary to trade off efficacy against other factors, for example compatibility with organic farming systems or reduced environmental persistence.'
- 'Biologically derived chemicals are often poorly characterised and the purity and amounts of contaminants are not readily available.'
- 'Residue studies are usually not required for biologically derived chemicals.'

Hence, in terms of the cost of satisfying the regulatory requirements for bringing a new blowfly control product to the market, the development of some biological-based products can have significant advantages over the development of new synthetic chemical products.

4.3.7.1 Plant extracts:

A number of plant extracts have been reported to show some activity against larvae of the sheep blowfly (either *L. cuprina* or *L. sericata*) or other blowfly species:

- plant-derived essential oils (Callander and James 2012; Khater et al. 2018; Chaaban et al. 2019a, b)
- plant extracts (Siriwattanarungsee et al. 2008; Mukandiwa et al. 2012)
- alkaloids (Green et al. 2002).

However, high levels of the extracts or oils were required to reduce larval survival to near zero in these studies. This is illustrated by the study of Callander and James (2010), who showed that tea tree oil at a concentration of 0.9 % caused 99% mortality of first instar sheep blowfly larvae *in in vitro* assays (LC_{99}). This level of active agent is 800 - 3,000-fold higher (in terms of the relative mass of material) than the levels of ivermectin or spinosad required to kill 95 % of the larvae (IC_{95}) in the same assay format (Levot and Sales 2002, Levot et al. 2002). The oil was not active against third instar larvae *in vitro*, indicating that that it would not be suitable for killing larvae at existing strike sites (as a dressing treatment). However the authors note that addition of tea tree oil to wound treatments may aid in wound protection and myiasis resolution by preventing oviposition by *L. cuprina* adults, insecticidal action against *L. cuprina* eggs and larvae, stimulating larvae to leave the wound and through antimicrobial and anti-inflammatory properties that aid in wound healing. Eucalyptus oil is included as an active ingredient along with an organophophate insecticide in at least three blowfly dressings, (Two formulations of Defiance 'S' Mules and Wound Dressing, Zoetis Australia Pty Ltd and Mules and Mark Blowfly, Dressing Elanco Australasia Pty Ltd) although the exact activity it provides is not specified.

4.3.7.2 Arthropod venoms:

Extracts prepared from spider venoms, and peptides purified from the venoms of spiders and predatory ants, have been reported to show activity against the adult life stages of the sheep blowfly (Ikonomoppoulou et al. 2016; Touchard et al. 2016; Smith et al. 2017; Guo et al. 2018). However,

these studies have involved the treatment of adult flies only, with the venom extracts or peptides administered either by injection or feeding. Hence, the potency of the venom components against the larval life stages remains unknown. Importantly, activity of venom peptides administered to adult flies by feeding was much less than by injection (Guo et al. 2018). As suggested by the authors, this lack of activity after ingestion was most-likely due to degradation by protease enzymes in the gut of the fly. Such degradation by proteases in both the saliva and midgut of blowfly larvae is also likely to have a major negative impact on the use of other peptides for blowfly control.

4.3.7.3 Ribonucleic acid molecules (gene-silencing):

RNA interference (RNAi) is a molecular biology technique for silencing the expression of genes in an organism. Double-stranded ribonucleic acid (dsRNA) molecules initiate a process in which specific genes within an organism are 'switched off'. RNAi has become a common mechanism of gene silencing in many model organisms. It is widely used as a drug target validation tool; the silencing of a specific gene leading to death of the organism provides evidence that the protein product of this gene is essential for the survival of the organism, and hence that targeting of the protein using a drug may be lethal. An alternative use of gene silencing is in the use of dsRNA molecules themselves as a therapeutic rather than as a means to identify potential drug targets. In this way, dsRNA molecules would act as an insecticide. The use of dsRNA molecules has a number of the advantages associated with biological control agents compared to synthetic chemicals (described on page 33): less persistency in the environment, and specificity for the insect pest (as the molecule is based on the gene sequence of that insect species) with no effect on non-target insect species. There has been a great deal of interest in this approach to insect control in recent year, with a focus on foliar application of dsRNA molecules for the control of insect pests of crops (Miguel and Scott., 2015; Liu et al. 2020; Christiaens et al. 2020).

However, there are significant differences in the interactions of different insect species with dsRNA molecules, and hence in the ability of the dsRNA to elicit a gene-silencing response. Some insects respond to ingested dsRNA, whereas others do not. These differences relate largely to species differences in the uptake of dsRNA into the cells of the insect, the spreading of the gene-silencing effects within the insect, the levels of enzymes that degrade the dsRNA (nucleases) produced by the insect, and pH conditions (acidity) in the insect gut. Recent work at the CSIRO has looked at whether ingestion of dsRNA by blowfly larvae resulted in any inhibition of larval development (Andrew Kotze, unpublished data). dsRNA molecules were designed using blowfly gene sequences, and fed to 1st instar larvae, and their development monitored over 4 days. The genes targeted were selected based on the known effects of dsRNA in inhibiting the development of other insect species (mostly caterpillars and beetle larvae). The dsRNA molecules were encapsulated in various matrices in order to protect them from nuclease enzymes. None of the dsRNA molecules were able to inhibit the development of blowfly larvae. There are a number of likely explanations for this, particularly, the activity of nuclease enzymes in degrading the dsRNA molecules, both external to the larvae by nucleases in the saliva secreted onto their food material as the larvae fed, and nucleases within the larval gut once ingested, and the low pH of the blowfly gut. It is likely that these factors will act against the usefulness of this approach to blowfly control. The work did not however closely examine the interaction of the blowfly nuclease enzymes with the dsRNA in the various encapsulation matrices, and hence, the protection and the matrix-release characteristics were not optimised. Further work to identify optimal encapsulation and release technologies may be warranted. However, importantly, as highlighted in the insecticide section of this report, commercial interest in this area of blowfly control will be adversely affected by the lack of market size for any blowfly-specific control.

4.3.8 Conclusions

To date, no natural biocontrols that appear to exert significant regulating influences on sheep blowfly populations have been identified, and the population dynamics and life history of *L. cuprina* would seem to present significant difficulties for any classical biological control agent to exert meaningful levels of control.

Innundative use of entomopathogenic bacteria (particularly *Bt*) and fungal biopesticides have shown some promise, and are attractive option in terms of 'natural' control, but to date periods of protection have been too low (either due to environmental degradation or failure to migrate as wool grows) to make them a viable option. The use of entomopathogenic viruses for flystrike control is an area yet to be explored but would appear likely to suffer similar difficulties to bacteria and fungi.

The potential for using bacteria or fungi that can actively grow in the fleece (for example *Bt* or a bacterium engineered to carry *Bt* toxins) may present an area of opportunity but investigations in this area to date have not yielded encouraging results, and the use of genetically modified pathogens may face regulatory and marketing barriers. Further opportunities in this area may lie in the identification of novel biocontrol organisms, as illustrated by the recent MLA-funded project that has identified soil-borne bacteria with acaricidal activity, with preliminary evidence indicating an ability of provide some degree of tick control following topical application to cattle (MLA, 2020).

Biopesticides based on plant extracts or other natural toxins are a particular area of interest because of their natural and environmental credential, but generally provide limited protection times that limit their use in practice. The possibility of extending periods of protection using microencapsulation or nanotechnology or other 'smart' formulations to extend protection periods should be investigated.

Some of the issues that may act against the development of biologicals for blowfly control, for example, lower efficacy compared to current chemicals, and shorter protection periods offered by less stable biologicals, may be lessened as resistance to the currently-available blowfly control chemicals impacts significantly on their usefulness, and the need for alternative agents becomes more urgent.

4.4 Novel delivery of flystrike prophylactics and therapeutics

4.4.1 Controlled-release technologies

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. However, there is evidence that the development of resistance is reducing protection periods provided by the most commonly flystrike chemicals (see section 4.1) and the high cost of developing and registering new chemical actives together with the relatively small size of the sheep flystrike market is slowing the development of new chemicals for flystrike control

State-of-the-art formulation provides a potential solution to address the issues with current sheep parasite control actives and recent years have seen significant advances in the area of controlled release technology. A number of long-acting injectable formulations for internal and blood feeding parasite control on livestock and companion animals are now registered, and controlled release (CR) devices such as rumen capsules for helminth control, polymer matrix ear tags for buffalo flies in cattle, and flea collars for parasite control on cats and dogs, have become major methods for

providing extended protection against animal parasites. In addition, the use of nanotechnology for medical, veterinary and agricultural application is rapidly increasing with the number of patents involving nanoparticles in these areas increasing from near zero in 1990 to close to 40,000 in 2020. (Chariou et al. 2020). However, with the exception of ruminal capsules and long acting macrocyclic lactones (MLs) for the control of gastrointestinal parasites, there has been limited use of CR technology against sheep ectoparasites.

There has been interest in the use of CR technology for sheep parasites since the at least the mid 1980s. Appleyard et al. (1984) showed that polymer matrix tags impregnated with cypermethrin gave season-long protection of sheep against head flies *(Hydrotaea irritans*), which are a significant pest of sheep production enterprises in the UK. Tags impregnated with insecticide were also shown to give varying degrees of control against ectoparasites which infest sheep at sites away from the head and Lloyd et al. (1984) showed that insecticide tags could eradicate sheep ked *(Melophagus ovinus* L.) if applied immediately after shearing. In Australia, tags containing 8.5% cypermethrin applied to louse-infested sheep with 2 months wool were found to reduce sheep louse numbers by 85% in comparison with controls at 38 weeks post treatment, and reduced lice to non-detectable levels in 67% of sheep when applied immediately after shearing. However, the period of protection provided against new infestations of *B. ovis* by the tags was not significantly different to that provided by a commercial cypermethrin backline treatment (James et al. 1989, 1990). The better effect when applied to infested sheep than prior to infestation was thought to be due greater pruritic activity transferring more chemical onto the wool and perhaps enhanced spread of chemical active on louse-infected skin (James et al. 1990). In studies against poll strike, when the rams were exposed to high populations of *L. cuprina* in an exposure house up to 18 weeks after treatment, 3.3% of rams treated with diazinon tags were struck compared to 57.1% treated by diazinon jetting (James et al. 1994). The possibility that tags might be able to administer enough chemical onto the fleece to control breech or body strikes was also considered, but although the tags rubbed insecticide onto the wool close to the strike, the concentrations of chemical delivered was considered unlikely to provide significant control.

CR capsules containing ivermectin and cyromazine have also shown potential for ectoparasite control in sheep (Anderson et al. 1989; Rugg et al. 1997; Rugg et al. 1998; James et al. 2017). Studies on the effect of ivermectin rumen capsules designed for use in GI parasite control, showed that although ivermectin capsules provided extended protection against breech strike, only moderate protection was provided against bodystrike (Rugg et al. 1998). In pen trials in the same study, where sheep were induced to scour and then exposed to high artificial challenge, the ivermectin capsules were 100% effective in preventing breech strike and number of bodystrikes was reduced by 35%. A similar pattern was seen in field studies where breech strike was reduced by 86% and there was a 27% reduction in body strike. The superior performance against breech strike was attributed to excretion of ivermectin in the faeces of scouring sheep. It was noted by the authors that although scouring and faecal soiling of the wool is a major predisposing factor for breech strike, urine staining is also important, and strikes in urine stained areas may have been responsible for the incomplete protection against breech strike in the field studies. It was also shown that ivermectin capsules could provide 100% protection against implants with Old World screwworm for at least 12 weeks (James et al. 2017) and were 100% effective against sheep nose bot when tested in a western NSW Merino flock with a 60% prevalence of nose bot (Rugg et al. 1997). It is notable that these results were with a capsule designed for the control of gastrointestinal parasites and it appears that capsules purpose-designed for control of flystrike that provided higher levels of ivermectin in the serum could give more complete protection against all forms of strike.

A particularly interesting application of capsule technology where release rates were designed to give flystrike-active concentrations of cyromazine in the serum was described by Anderson et al. (1989). Following calibration to determine appropriate release rates, they were able to achieve up to 12 weeks protection. An added attraction of this capsule is that release of cyromazine from the capsule gave an approximately 'square wave' profile plasma concentration, providing very steep decay tails. Thus, the intensity of selection for resistance to the chemical is low compared with that from the usual topical applications where extended decay tails are usually observed. The other attraction of systemic delivery of insecticide is that chemical is delivered at measured rates to all sites on the body, reducing the possibility of protection breakdown due to uneven application, a common issue with topical treatments. The authors suggest that these capsules would be particularly applicable for the early season treatment strategy as suggested by McKenzie and Anderson (1990) because they would also guard against covert strikes, thought to be important in early season build up and population maintenance through low strike periods (Wardhaugh and Dallwitz 1984, Anderson et al. 1988).

In preliminary studies examining the potential of a 'strategic release' approach to flystrike formulations, James et al. (1994) investigated the effectiveness of encapsulation insecticide in starch xanthate and starch borate formulations. Diazinon was used as the active ingredient in these formulations for experimental purposes. The starch xanthate formulations which had previously been found to improve the effectiveness of herbicides, nematicides and feed-through insecticides for control of flies in poultry manure (Shasha 1980) release insecticide when moistened. As flystrike is always associated with moisture, whether it be from the predisposing causes or from plasma fluids released from strikes, it was hoped that the formulation would remain inert in the fleece at times of low strike risk and only release insecticide when moisture was present, thereby giving extended protection. Although the starch xanthate formulation gave significantly longer protection than conventional formulation in a number of studies, the effects were inconsistent and this appeared to be due to difficulties in achieving even coverage with the particulate formulation (James et al. 1994, James PJ and Cooper DJ 'Controlled Release Insecticide Systems for Sheep Ectoparasite Control' Final report; Wool Research and Development Corporation Project DAS11, 1993).

A further novel usage of CR technology for myiasis control is the use of a sustained-release ivermectin that has been used for the treatment of wound myiasis in zoo- housed animals to reduce the need for recapturing and anaesthesia of affected animals for retreatment (Avni-magen et al. 2018) The formulation was based on an ivermectin formulated with aminomethacrylate copolymer, polyethylene glycol and hydroxypropyl cellulose which dried on application to clear adherent mobile coating, and showed insecticidal efficiency for up to 15 days. Only 1 of 11 animals treated with the varnish required retreatment whereas 13/17 animals treated by ivermectin injection required retreatment. This work was conducted in Israel and the species responsible was not specified, however is almost certainly not *L. cuprina* and was more likely due to screwworms, which are much more invasive than *L. cuprina*. Most *L cuprina* strikes heal well even without the application of a pesticide if the maggots are removed and the wool clipped away to allow the strike to dry. It seems unlikely that a similar treatment would confer a significant advantage under Australian conditions.

4.4.2 Nanotechnology

In recent years there has been exploding interest in the area of nanotechnology, with approximately 60,000 papers published in the literature since the early 1990's (Chariou et al. 2020). Controlled release technologies can be more efficient in delivering active ingredient with reduced likelihood of non-target effects such in the environment, potential for residues and operator exposure in carefully

designed systems, and lower likelihood of selection for resistance. Notably, from a practical perspective, they can usually be applied using conventional application equipment.

There are now myriad designs of nanoparticle formulations designed from a wide range of categories that can be classified in a range of different ways depending on the end use. For veterinary application alone they can be divided into polymeric nanoparticles, liposome nanoparticles, micellar nanoparticles, dendrimer nanoparticles and metallo nanoparticles (Bai et al. 2018). This classification refers mainly to nanoparticles designed for systemic administration. When designed for systemic absorption, the ability of the particles to cross the skin barrier may be important, but when designed for topical application against ectoparasites different factors come into play as it may be important to design the application to prevent skin penetration. Importantly, many of these are completely inert or biodegradable, with high safety profiles.

Most of research and development of nanoparticles to date have been in the area of medical technology, representing approximately 93% of papers to date. Interest in agricultural and veterinary uses, which make up 4% and 3% of the publications, respectively, has emerging later, in the 2000's (Chariou et al. 2020). A scan of the veterinary nanoparticle formulations approved for use or undergoing clinical trials indicates most are targeting various carcinomas and the authors note that most of the applications also have implications for human uses. Notably there are few formulations for parasites amongst these (Bai et al. 2018, Chariou et al. 2020) one exception being the recent report of the use of elagic acid loaded nanoparticles against the protozoan cattle blood parasites *Babesia* and *Theileria* (Beshbishy et al. 2019).

However, chemical pesticides are well suited to nanoformulation because they tend to be small molecules and they are usually effective at very low doses, and there are now significant numbers of nanoencapsulated pesticide compounds, including insecticides, registered or in development for agricultural use. Nanoformulation can markedly increase the efficiency of agricultural pesticides and pesticides for ectoparasite control by preventing photodegradation and evaporative loss in the case of volatile active ingredients, and extending periods of protection through providing controlled release. It is notable that a number of nanoformulations containing insecticides that have already been used for ectoparasite control contain compounds known to be active against Diptera, for example imidacloprid (Guan et al. 2008, Adak et al. 2012), methomyl (Yin et al. 2009) and emamectin benzoate (Shang et al. 2013). Guan et al. (2008) demonstrated that formulation of imidacloprid microcrystals in polysaccharide nanocapsules could increase the release time by up to 8x in comparison with uncoated imidacloprid, and Adak et al. (2012) showed that nanoencapsulated CR imidacloprid gave superior protection of soybean pests in comparison to commercial formulations. Shang et al. (2013) showed that encapsulation of emamectin benzoate (a macrocyclic lactone drug which is known to have poor photostability) greatly improved both photostability and insecticidal effects.

Nanotechnology can also be effectively applied to more complex biopesticides and essential oils and may be of particular use for 'natural pesticides' where practical use is currently limited by high volatility or low photostability. For example, Yang et al. (2009) found that nanoparticles loaded with garlic oil tested against red flour beetle, an important stored grains pest, gave 80% control over five months, in comparison to only 11% from free garlic oil at the same concentration. In this case where the formulation was applied indoors in grain storage this was thought to be due to controlled release of the oil. Notably, garlic oil is also known to have a range of activities against blowflies (Bedini et al. 2020). Lao et al. (2010) showed that formulation in chitosan nanoparticles could also be successfully applied to the water-soluble botanical pesticide rotenone, which is very prone to photodegradation. In addition, although not yet tested with insects, formulation in nanoparticles

may also be able to improve the effects of biological molecules with small interfering RNAs providing 6 days against viral infection when applied directly onto leaves, but approximately 20 days when delivered on clay nanosheets (Mitter et al. 2017). More recently silica nanoparticles designed with 'pollen like' topology and surface whiskers to increase adhesion when applied to wool were shown to reduce chemical loss and protect against chemical photodegradation and rainfall leaching, and provided increased periods of protection in comparison to conventional formulations (James et al. unpublished data)

4.4.3 Conclusions

CR technology can provide extended periods of protection, have a number of advantages over traditional methods, and may provide a means of utilising additional pesticide actives that do not provide practical periods of protection when applied in conventional 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. Therefore, initial doses need not be as large, thereby reducing the risk of tissue residues, environmental contamination, operator exposure and other off- target effects. Some systems can also deliver 'square' release curves without extended decay tails thus reducing selection for resistance. In addition, CR systems may enable the use of 'softer' chemistries, often favoured because of their rapid degradation in the environment and consumer preference. A wider choice of insecticidal actives would be valuable in providing additional chemical options in planning insecticide usage programs designed to minimise resistance development.

4.5 Vaccination against flystrike

4.5.1 Flystrike vaccine research conducted in the 1980s and 1990s

A great deal of work to develop a vaccine for flystrike was undertaken by CSIRO and the University of Melbourne in the late 1980s, the 1990s, and early 2000s.

The University of Melbourne group focused on antigens that were recognised by the sheep immune system during natural blowfly infestations. The CSIRO group focused on antigens that were able to produce antibodies that inhibited larval growth *in vitro*. Most of these CSIRO antigens were gut-associated ('hidden antigens') and poorly immunogenic during natural infection.

A number of sheep trials were conducted using native antigens recovered from blowfly larvae or recombinant proteins (produced in bacterial, insect and yeast systems).

The effectiveness of the vaccinations were assessed using *in vitro* assays for most of the sheep trials, and also with *in vivo* measurements of larval growth or protection against flystrike in some cases:

- *In vitro*: larvae were fed on artificial diet containing serum recovered from vaccinated (and control) animals. Effects on weight gain over short periods of time (usually 20 hrs), and on larval survival at this same time point, were assessed. In some cases, observations were made on subsequent development and survival of the larvae.
- *In vivo*: the growth of larvae and their ability to establish wounds at larval implant sites on vaccinated (and control) sheep were measured.

Summary of trial outcomes:

The major outcomes of the various studies in terms of effects on growth of blowfly larvae *in vitro* and *in vivo*, and on their ability to establish strikes at implant sites *in vivo* (only assessed in a couple of studies), are summarised below and in Table 1.

- *In vitro*: A number of trials showed that serum from vaccinated animals was able to inhibit the growth of larvae *in vitro*. However, the effect on larval growth was only temporary, with most or all of the larvae completing their development after the initial slow phase. Most effects on larval growth *in vitro* were below the threshold required for any significant larval mortality to occur (threshold is described in more detail in section 4.5.2.1 below). The exception to this was a report by Tellam and Eisemann (1998) in which the inhibition of larval growth *in vitro* was just above this threshold, with larval weights reduced by 84 % after short term feeding (20 hrs) on serum from vaccinated animals, with 35 % mortality. The subsequent ability of the remaining 65 % of the population to grow after this time point was not measured, and no *in vivo* experiment was conducted.
- *In vivo*: In most cases, the effects on growth of larvae on sheep (*in vivo*) were even less marked than *in vitro*. There were however a couple of exceptions to this:

  1) Bowles et al. (1987) found that all larvae died at implant sites on 3/10 vaccinated animals; however this also occurred on 1/10 control animals.

  2) Bowles et al. (1996) showed an 86 % reduction in strikes after 48 hours at larval implant sites on vaccinated animals (n=3 animals) compared to controls (n=4) in one trial, and a 67% reduction in another trial (n = 8 animals per group);

The research program ended in the early 2000s, with no vaccine commercialised.

Table 1: Antigens and outcomes for blowfly vaccine studies (1981 – 2002)

(note: ES = excretory / secretory products = proteins collected from a liquid medium in which larvae have been incubated for 48 hrs)

Reference	Antigen	Outcomes		Comments
		In vitro	*In vivo*	
O'Donnell et al. 1981	Extract of 3rd instar larvae	15 – 20 % reductions in larval survival	No effect on larvae	No significant effect of vaccination
Bowles et al. 1987	ES from 2nd instar	Not tested	Vaccine administered intradermally: no effect on larvae Vaccine administered intranasally: all larvae died on 3 / 10 treated animals, and all larvae also died on 1 / 10 control animals.	complete inhibition of larvae on 3 / 10 treated animals; however, larvae also completely inhibited on 1 / 10 control animals
East et al. 1992	extract of 1st instar larvae	Larval growth decreased by up to 57 % at 20 hrs	Larval weight reduced by up to 35 % at 50 hrs	short-term (20 hrs) effect on *in vitro* larval growth; No correlation between antibody levels and reductions in growth across different adjuvant treatment groups
Johnston et al. 1992	Extract from whole 2nd instars, or guts	Larval weight reduced by up to 23 % at 20 hrs	Larval weight reduced by 26 % at 20 hrs, and up to 60 % at 44 hrs; but no larval mortality, and no weight difference at end of feeding stage	Temporary effect on larval weight gain, followed by normal development, no mortality
East et al. 1993	Peritrophic membrane extract	Larval weight reduced by up to 50 % at 20 hrs	Larval weight reduced by up to 50 % at 20 hrs, and 35 % at 50 hrs; No larval mortality	Reductions in weight gain, but no mortality
Tellam et al. 1994	i)purified protease enzymes ii) crude ES extract	i) no effect on larval growth ii) larval weight reduced by 50 % after 20 hrs (weight only reduced in assays with	i) and ii) no effect on larval growth	No significant effect of vaccination

Reference	Antigen	Outcomes		Comments
		In vitro	*In vivo*	
		serum from 2 out of 4 treated animals)		
Bowles et al. 1996	Extracts from 1st instar larvae		Trial 1: After 48 hrs, 86 % reduction in strikes in treated animals, and 85 % reduction in larval weight; inhibition of wound formation in 8 out of 9 sheep. Trial 2: 67 % reduction in strikes in treated animals at 48 hrs, and larval weight reduced by 30 %.	significant protection from flystrike *in vivo*; serum antibody levels did not correlate with protection from strike
Casu et al. 1997	Peritrophin-95 (purified from larval extracts)	Larval weight reduced by 60 % after 20 hrs; no effect on larval survival at 20 hrs; no effect on pupation rate (ie larvae completed their development after initial delay)	Not performed	short-term (20 hr) *in vitro* effect; larvae then completed their development as normal
Tellam and Eisemann 1998	Extracts from 1st instar larvae; the most effective fractions contained peritrophic membrane proteins	larval weight reduced by 84 % at 20 hrs; 35 % larval mortality at 20 hrs	Not performed	Significant short-term (20 hr) *in vitro* effect
Tellam et al. 2001	Native and recombinant peritrophic membrane protein (PM95)	larval weight reduced by 60 % at 20 hrs using native antigen; no effect on survival of larvae at 20hrs; larval weight reduced by < 20% with	Not performed	Short term *in vitro* effect on weight with native protein, however no larval mortality. Recombinant protein was ineffective

Reference	Antigen	Outcomes		Comments
		In vitro	*In vivo*	
		recombinant antigen		
Bowles 2001	Various native and recombinant peritrophic membrane proteins, and other non-specified proteins (n =12 separate antigens)	Larval weight reduced by up to 66% with some native proteins; no significant reduction with recombinant proteins	No statistically significant protection by native or recombinant proteins	Recombinant proteins less effective than native proteins *in vitro*; All recombinant and native proteins ineffective *in vivo*
Colditz et al. 2002	Recombinant peritrophic membrane proteins	Not performed	Not performed	

4.5.2 Biological factors impacting on flystrike vaccination

4.5.2.1 *In vitro* versus *in vivo* effects on growth of blowfly larvae

Many of the studies in the 1990s demonstrated an effect on larval growth *in vitro* after feeding on serum from vaccinated animals. These effects were often significant in a statistical sense, however, not in a biological sense in terms of flystrike as they were only temporary in nature. The larvae grew as normal after an initial slow phase. Larval growth was inhibited at early time points (usually 20 hrs) but then the larvae continued to grow and pupate. Hence, such growth inhibition is of little biological significance in terms of preventing flystrike. The larvae would be able to establish strikes, or exacerbate existing strikes, *in vivo*, if inhibited to the same degree at an early time point.

East and Eisemann (1993) reviewed a number of studies on the effects of serum from vaccinated animals on larval growth *in vitro*. They concluded that larval size at 20 hrs in *in vitro* experiments needs to be no more than 20 % of controls (80 % inhibition of growth) in order to prevent the larvae developing fully to the pupal stage. Most of the studies conducted in the 1990s reported larval inhibition at much less than this 80% level. One study did report *in vitro* inhibition of larval growth of > 80 % at 20 hrs, alongside mortality of 35 % (Tellam and Eisemann 1998), however, the study did not continue to monitor weight gain or mortality at later time points, and no *in vivo* assessment was performed.

The required threshold of larval growth inhibition becomes even greater when translating *in vitro* experiments to an *in vivo* infection. Eisemann et al. (1993) showed that larvae at infection sites on sheep ingested much less antibody than larvae feeding in *in vitro* assays on artificial medium supplemented with serum from vaccinated animals. The larvae in their experiment had 66% less antibody uptake when feeding on sheep. Hence, while a threshold of > 80% inhibition of the growth in early stage larvae may be required to prevent subsequent development *in vitro*, the threshold

may actually be much higher when assessing translation from *in vitro* experiments to the prevention of larval growth *in vivo* as these larvae will ingest much less of the antibody.

4.5.2.2 Antibody-mediated or cellular response required?

There has been some debate as to whether vaccination against flystrike should be aiming to elucidate a humoral or a cellular response if protection is to be provided. Simple definitions of the two types of response:

Humoral (antibody-mediated) immunity: mediated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. With respect to flystrike, the molecules of interest here are antibodies produced in sheep in response to exposure to blowfly proteins in a vaccine.

Cellular immunity: the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to antigen. Various cell types produce cytokines which activate mononuclear phagocytes, recruit neutrophils to the infection site, and induce epithelial responses that act against foreign organisms.

Most of the work conducted in the 1990s was centred on generating high levels of serum antibody to act against the blowfly larvae. However, the most successful *in vivo* studies from that time (Bowles et al. 1996) found that protection against flystrike after vaccination was associated with cellular responses rather than antibody- mediated responses. Bowles et al. (1996) reported significant levels of protection in sheep immunised with 4 larval antigens, however, antibody titres were not correlated with protection. On the other hand, there was a significant presence of a number of different immune cell types at the site of challenge in animals that were protected from flystrike. This suggested a role for these cells in prevention of strikes.

In reviewing these early vaccine trials, Elkington and Mahoney (2007) suggested that future efforts in flystrike vaccination should be directed at generating a cellular rather than an antibody-mediated response. They described the failure of previous efforts that relied upon the generation of humoral immunity through the uptake of antibodies, as well as basic blowfly biology factors that affect their interaction with antibodies. They highlighted the lack of correlation between antibody levels and protection against flystrike in some earlier *in vivo* studies (East et al. 1992, and Bowles et al. 1996), limitations in the quantity of antibody likely to be present at the infection site at the time of wound initiation, and degradation of antibodies at the wound site and in the blowfly gut (examined in more detail below).

4.5.2.3 Stability of antibody at the blowfly wound site, and in the blowfly gut

i) at the wound site on sheep:

Interaction of host-generated antibodies with their target protein in blowfly larvae would require the antibodies to remain active following secretion into the infection site. However, the wound site would be expected to contain high levels of proteolytic enzymes derived from two sources; firstly, the serum exudates produced by damaged host cells, and secondly, in the excretory / secretory products of the blowfly larvae. This second source may be expected to be particularly damaging to antibodies as it contains many protease enzymes that act in the partial digestion of host material prior to ingestion by the blowfly larvae.

Sandemann et al. (1995) examined this issue by measuring the time course of degradation of antibodies at blowfly infection sites on sheep. They found that 60 % of the antibody in serum

exudates at the infection sites was degraded by 6 hrs after infection, indicating the presence of very active proteolytic enzymes. The level of degradation was 68 % after 48 hrs. An examination of the sizes of the breakdown products indicated that the major source of the enzyme activity was enzymes secreted by the blowfly larvae as opposed to host-derived enzymes. The authors concluded that their findings could have two opposing potential impacts on vaccine development:

- Negative: antibody degradation represents a significant barrier to the required uptake of high levels of antibody by blowfly larvae if their development is to be inhibited.
- Positive: the authors suggested that the fragments that are produced as a result of proteolytic degradation of the antibodies may still be active, and hence may still bind to their blowfly antigen targets. No evidence for this retention of activity by degraded antibodies was presented.

ii) within the blowfly gut:

A second aspect of the stability of antibodies is their degradation within the gut of blowfly larvae following ingestion. Eisemann et al. (1995) showed that ingested antibody remained intact in the anterior portion of the midgut but was degraded significantly in the mid-section of the midgut. This degradation was thought to be due to the action of acid protease enzymes in the low pH (acidic) environment of this mid-section. Hence, this study indicates that ingested antibody targeting the peritrophic membrane would likely remain intact in the anterior section of the midgut and hence bind to its protein target on the membrane, but then be liable to degradation as membrane secretion continued (from the cardia at the anterior end of the midgut) and the section of membrane with antibodies attached moved to within the mid-section of the midgut. The level of degradation would depend on how accessible the antibody bound to the membrane surface is to the protease enzymes in the gut. Antibody that had failed to bind to the peritrophic membrane in the anterior section, and had therefore moved through the gut lumen to the mid-section, would be liable to degradation by these protease enzymes.

The stability of antibodies in the blowfly gut is also important when considering the potential for blowfly vaccination given the use of antibodies targeting gut antigens as a proven means for vaccination against the cattle tick (TickGARD®, Gavac®). As highlighted by Elkington and Mahony (2007), differences in the feeding mechanisms of blowflies and ticks will act against this approach to vaccination in blowfly larvae. In ticks, digestion occurs by processes of phagocytosis and pinocytosis, in which the gut cells engulf solid particles or liquid droplets, respectively (Akov, 1982). Hence, ingested antibodies gain access to the target gut cells. In contrast, in blowfly larvae, digestion occurs in an extracellular environment in the gut, followed by absorption of nutrients across the peritrophic membrane and then across the gut epithelium. Hence, ingested antibodies will be exposed to digestive enzymes in the lumen of the blowfly larval gut before they are able to make contact with gut cells.

4.5.2.4 Immunomodulation by blowfly larvae

There is evidence that blowfly larvae interfere with the ability of the host to mount an effective immune response. Kerlin and East (1992) described the suppressive effect of larval ES products on ovine lymphocytes. Lymphocytes co-incubated with ES showed a significantly reduced ability to proliferate in response to mitogens. In addition, co-injection of ES and equine myoglobin into sheep reduced the production of anti-myoglobin antibody by the sheep compared to animals that had received only the equine myoglobin. Elkington et al. (2009) subsequently described a protein in ES that inhibited lymphocyte proliferation, and named it blowfly larval immunosuppressive protein

(BLIP). They demonstrated that BLIP binds to the surface of ovine lymphocytes and leads to changes in the early events involved in lymphocyte activation. They suggested that this was a means used by the larvae to inhibit the sheep immune response.

This immunomodulation has potentially very significant implications for the development of a flystrike vaccine. It is possible that even if potentially-protective responses could be generated though vaccination, the blowfly larvae may be able to supress these responses to such an extent as to allow the infection of the host animal to proceed as normal. Hence, Elkington and Mahony (2007) suggested that the targeting of the blowfly immunomodulation mechanism itself may be a useful component of any strategy for developing a flystrike vaccine.

4.5.2.5 Native vs recombinant antigens

An issue that impacts antiparasitic vaccines is the difference in immunological activity between native antigens (recovered directly from the target parasite using biochemical techniques) and recombinant antigens (produced in laboratory cell culture systems). Often, immune responses observed in response to exposure to native antigens, do not occur, or are greatly reduced, in response to recombinant antigens. The native and recombinant antigens differ in structural properties associated with the folding of the protein and post-translational modifications that occur when the protein is produced in the target parasite, but not when it is produced in the laboratory cell system, namely, glycosylation (addition of specific sugar molecules to the protein). Yet, recombinant antigens are required if a vaccine is to be produced in a cost-effective manner, as recovery of native antigens from larvae would not be practical for blowfly vaccine production. An exception to this is the Barbervax® vaccine for *Haemonchus contortus* which contains native antigens recovered by the processing of adult worms recovered from the abomasa of sheep at abattoirs. However, such a production system utilising blowfly larvae would not be practical for cost-effective production of a flystrike vaccine.

Tellam et al. (2001) addressed this issue directly by comparing the response to a native protein recovered from larvae (peritrophin-95) and recombinant versions of the protein produced in both bacterial and insect cell culture systems. Serum from animals treated with the native antigen inhibited larval growth in vitro by 60 % at 20 hrs (in agreement with their earlier study, Casu et al. 1997), while the two recombinants resulted in < 20 % inhibition of larval growth. Bowles (2001) described a series of 12 separate trials conducted by the University of Melbourne and CSIRO between 1996 and 1999 comparing native and recombinant antigens. While sera from sheep treated with some of the native antigens inhibited larval growth *in vitro*, the sera from sheep vaccinated with each of the recombinant antigens did not inhibit larval growth significantly.

4.5.3 Conclusions:

While the notion of vaccination against flystrike is appealing, there are a number of biological factors that will make this a difficult goal to realise. Work was conducted on flystrike vaccination in the 1980s and 1990s, however, no vaccine was commercialised. One report did describe a small-scale vaccination trial in which significant levels of protection were observed (Bowles et al. 1996). The protection observed in this experiment did not correlate with antibody levels, suggesting that a cellular immunity pathway may have been responsible for the observed protection.

A number of investigators have described aspects of the sheep / blowfly interaction that will impact on the ability for vaccination to provide protection against flystrike:

- Temporary nature of the inhibition of larval growth observed in *in vitro* and *in vivo* experiments; larvae feeding on serum recovered from vaccinated sheep, or feeding directly on vaccinated sheep, grew more slowly than controls for a period of time (usually assessed at 20 hours), but then grew to the fully-grown stage as normal, and hence would be able to establish strikes as normal.
- Inability of antigens produced in bacterial recombinant systems to elicit an effective immune response.
- Inability of antigens produced in yeast and baculovirus systems to elicit an effective immune response (only limited numbers of antigens were examined in these experiments).
- Evidence for the need to elicit a cellular rather than antibody-mediated immune response.
- Instability of antibodies at the wound site, and in the gut of blowfly larvae (liable to degradation by sheep- and blowfly-generated protease enzymes).
- Production of immunomodulatory molecules by blowfly larvae; these will act to suppress any immune response that may be generated by vaccination.

4.6 Vaccination against fleece rot

4.6.1 Previous work on fleece rot vaccination as a means to control flystrike

Fleece rot is considered to be a major pre-disposing condition for body strike in sheep. It has therefore been suggested that a means to reduce the incidence of flystrike would be to reduce the incidence of fleece rot through vaccination against the bacteria that cause the condition. The antibodies produced by the sheep in response to vaccination may be able to prevent the growth of the bacteria at the skin surface, and hence prevent the development of the dermatitic lesions and the fleece rot-associated odours that are thought to attract flies to the sheep.

The bacterium *Pseudomonas aeruginosa* has been shown to be the organism responsible for the green discolouration of fleece that is commonly seen with fleece rot (Burrell et al. 1982). On the other hand, *P. maltophilia* is associated with the brown to yellow type of fleece rot (MacDiarmid and Burrell, 1986). However, other bacteria are also observed in fleece rot (see section X.2.3).

A program of work on vaccination against fleece rot was conducted at CSIRO in the 1980s (Burrell, 1985, 1987, 1990). The work focused on vaccination against *P. aeruginosa* as a means to control fleece rot, and bodystrike. Major outcomes:

1) Burrell (1985):
   - Experimental challenges; a series of small-scale experiments (n=3 animals per group) showed that vaccination with *P. aeruginosa* could protect sheep from dermatitis and artificial flystrike challenges.
   - Field challenge: 26 merino lambs were vaccinated using a crude extract of *P. aeruginosa*, and were then run with 115 'similar' paddock-reared (control) sheep at Badgery's Creek, NSW. Over 120 m of rain fell in Nov-Dec, leading to a fly epidemic in mid-January coincident with the development of fleece rot. Of the 115 control sheep, 61 developed fleece rot, and 21 showed bodystrike. Of the 26 vaccinated sheep, none showed fleece rot or bodystrike. The experiment involved a group of vaccinated animals and a group of 'similar' animals as controls, rather than treatments across randomised animals from a single cohort.

2) Burrell (1987): Sheep were vaccinated with *P. aeruginosa*, followed by natural fly challenge; strikes occurred in 13.2 to 26 % of the animals across three groups of control sheep, compared to 2.5 to 6 % across 3 groups of vaccinated sheep.

3) Burrell (1990). A series of *P. aeruginosa* vaccination trials were conducted across 14 properties. Significant levels of protection against flystrike were seen on 4 properties where the homologous serotype of the bacterium was present, however, no protection against flystrike was seen on 10 properties where the natural challenges involved heterogenous serotypes of *P. aeruginosa* (serotypes described in more detail in section X.2.4).

4.6.2 Issues that impact on the potential use of a fleece rot vaccine for control of flystrike

4.6.2.1 Uncertainty about the obligate requirement of fleece rot for body strike to occur

While body strike most often occurs when fleece rot is also present, uncertainty remains as to whether fleece rot is a pre-requisite for strike to occur. The presence of wet wool and wet skin for a period of at least several days seems to be the only certain pre-requisite for body strike. That is, while prolonged wetting of the skin is associated with both body strike and fleece rot, it is uncertain

whether wetting alone, or wetting plus fleece rot, is/are required in order for flystrike to occur. Two aspects of the interaction between fleece rot and body strike are important:

1) Lack of proof that fleece rot odours are required to attract flies to sheep.

While the odours associated with fleece rot seem to greatly increase the attractiveness of sheep to female flies (eg. Emmens and Murray 1982), conclusive evidence that they are a requirement for flies to be attracted to sheep is lacking.

The nature of the odours that attract female flies to sheep was recently reviewed by James et al. (2019). They concluded that odour is very important in the attraction of flies. Sources of these odours included 'putrefactive' or bacterial odours associated with faecal and urine staining, fleece rot, and mycotic dermatitis, as well as sulphur volatiles from the cysteine component of wool, and odours emanating from existing strikes on the sheep (thought to be primarily of bacterial origin). The presence of these odours is consistently associated with wet wool and wet skin.

An example of the multi-component nature of the attractiveness odours is provided by Watts et al. (1981) who showed that odours recovered from wet wool impregnated with *P. aeruginosa* only attracted female flies to wool samples if the wool also contained the other normal components: wax, suint and epithelial debris. The odours recovered from wool that had been scoured (to remove these components) and then inoculated with *P. aeruginosa* was not attractive to flies.

2) Evidence that bacterial growth is not a requirement for initiation of strikes

The growth of first instar larvae in establishing a new strike site is presumed to depend on the larvae utilising host animal serum transudate as a source of nutrition. Some early reports concluded that bacterial growth (with release of proteolytic enzymes) was responsible for the formation of lesions on the skin surface, and the release of serum to provide nutrition for the 1st instar larvae (e.g. Arundel and Sutherland 1988). This conclusion was based to some extent on the observation that 1st instar larvae do not possess the mouth hooks that are present on 2nd and 3rd instars and are used to abrade the skin by these later life stages. Given that the 1st instars were assumed to not be able to generate serum transudate themselves, it was proposed that transudate as a result of bacterial growth was therefore a predisposing factor for strike initiation.

However, Mackerras (1936) and Sandeman et al. (1987) showed that, under artificial conditions, strikes could be initiated just by placing eggs or first instar larvae on wet skin, in the absence of fleece rot. Mackerras (1936) suggested that the maintenance of the skin in a wet state for several days (as is required for body strike to occur) was sufficient to irritate the skin to cause weeping of serum from the surface. Later reports described the formation of skin lesions and release of serum occurring as a result of prolonged wetting of the skin alone, without the requirement for bacterial growth (Hayman 1953; Nay and Watts 1977). In addition, Sandeman (1987) noted two sets of spines in the oral cavity of 1st instar larvae that may act to abrade the skin, and hence cause the release of serum transudate. This indicated that the 1st instar larva could likely abrade the skin in order to generate the required serum transudate at the skin surface.

Hence, two sources of serum, independent of the presence of bacteria, are apparent: firstly, transudate from skin that has been wet for a period of time, and secondly, the likely ability

of the larvae to rub spines against the skin surface to release the transudate. Mackeras (1936) and Sandeman (1987) suggested that such artificial infection experiments do not dismiss the role of bacteria in potentiating strike formation in the field, but do suggest that fleece rot is not a mandatory pre-requisite for body strike to occur.

4.6.2.2 Severity of fleece rot is not a predictor of susceptibility to forms of strike other than body strike

i) Raadsma et al. (1988) measured severity of fleece rot and incidence of flystrike (body strike and other forms) under artificial wetting and field rain regimes in two experiments; number of sheep = 168 and 176 wethers, respectively. Two aspects of the relationship between flystrike and wetting / fleece rot:

1) response to wetting: the incidence of body strike increased linearly in relation to the duration of wetting. On the other hand, the incidence of non-body strikes was not related to the duration of wetting (non-body strikes consisted of pizzle strikes 26 % of all strikes, as well as breech and head strikes (6 %).

2) severity of fleece rot: The incidence of body strike was closely related to the severity of fleece rot. On the other hand, the incidence of non-body strikes was not related to the severity of fleece rot.

ii) There is very little information available on the bacteria present on the sheep breech. The only two references to this that we could find in the literature are contradictory in terms of whether *Pseudomonas* is present: Bull (1931) examined skin folds after removal from lambs by the mulesing operation on a property in South Australia, and detected *Pseudomonas* and gram positive cocci resembling staphylococci. On the other hand, Burrell (1987) stated that 'the bacteria principally responsible for dermatitis in the breech area, unlike that on the body, are not *P. aeruginosa'*. Burrell (1987) described a study in which no *P. aeruginosa* was detected in at least 80 independent samples taken from ulcerative dermatitic lesions (both before and after fly strikes) over the summers of 1982-83 and 1983-84.

4.6.2.3 The presence of *P. aeruginosa* is not essential for fleece rot or body strike to occur:

i) Burrell and Macdiarmid (1986): a patent described the recovery of various *Pseudomonas spp*. from fleece rot and body strike lesions. *P. aeruginosa* was only present in a minority of fly strike and fleece rot sites, with other *Pseudomonas spp.* being more prevalent.

ii) Kingsford and Raadsma (1997): reported the occurrence of fleece rot and presence of *P. aeruginosa* in three surveys conducted between 1993 and 1995 on 2 group of sheep from 2 locations in NSW. Of a total number of sheep of 1568, the number with fleece rot score < 3 = 922 (considered to be 'non-affected'), number with fleece rot score > 3 = 646. Of these 646, the number that showed the presence of *P. aeruginosa* was only 92, that is, only 14 % of the sheep with fleece rot showed the presence of *P. aeruginosa*.

iii) Kingsford and Raadsma (1997) measured the number of sheep with body strike after natural field challenge at Camden NSW. Of a total number of sheep of 393, the number with a fleece rot score < 3 was 103 (considered to be 'non-affected'); none of these animals were struck; the number with a fleece rot score > 3 was 290, with 10 of these animals being struck. Of these 10 struck animals, 6 showed the presence of *P. aeruginosa,* 4 showed no presence of *P. aeruginosa.* Hence, 40 % of the struck sheep had fleece rot caused by an organism other than *P. aeruginosa.*

iv) Dixon et al. (2007) examined the bacterial populations present in samples recovered from the fleece rot-resistant and -susceptible lines of sheep (Trangie Research Station flocks) after artificial wetting. They identified the bacterial populations recovered from two susceptible

sheep that had shown fleece rot scores of 5 (maximum score) after 3 days of wetting. *P. aeruginosa* was not detected in fleece rot samples from the two sheep.

4.6.2.4 Existence of multiple *P. aeruginosa* serotypes

Up to 14 serotypes have been recognised among the *P. aeruginosa* samples recovered from sheep in Australia (Burrell and MacDiarmid, 1984; MacDiarmid and Burrell, 1986). Hence a vaccine targeting this bacterium as a means to control flystrike would need to be effective against multiple serotypes just to control this one bacterial species. Burrell (1990) showed that a *P. aeruginosa* vaccine only protected sheep from flystrike if the same serotype of the bacterium was present. This study found that a prototype commercial *P. aeruginosa* vaccine was able to provide some protection against flystrike on animals that showed the presence of the same serotype to which the vaccine was targeted on 4 properties, but provided no protection in animals on 10 other properties where the *P. aeruginosa* were from different serotypes.

In contrast, Burrell (1987) described some cross-serotype protection against dermatitis after vaccination with a single isolate of *P. aeruginosa* proved some protection against two other serotypes. Protection against flystrike was not assessed in this work.

4.6.2.5 Antibody response to fleece rot did not correlate with likelihood of flystrike

Dai (1997) reported on a fleece rot / flystrike trial at the Merck Sharp Dohme facility at Ingleburn, NSW. Eighteen sheep were seeded with *P. aeruginosa*, wetted artificially, and then exposed to flies. This process was repeated fortnightly for 14 weeks. Antibody response to *P. aeruginosa* was measured, and numbers of strikes on each sheep were recorded, after each round of exposure. Nine sheep maintained low *P. aeruginosa* antibody titres (denoted as low responders), and 9 sheep maintained high *P. aeruginosa* antibody titres (denoted as high responders). The average number of strikes per low responder sheep was 1.7 ± 0.3, and for the high responder sheep was 2.7 ± 0.6 (not significantly different). Hence, antibody response to *P. aeruginosa* was not related to likelihood of flystrike.

4.6.2.6 Lack of progress in developing a vaccine for human *Pseudomonas* infections

A great deal of effort has been put into developing a vaccine against *P. aeruginosa* in the field of human medicine as the organism causes multiple infections. However, while many vaccine candidates have been identified, no vaccine is currently available. Merahkou et al. (2018) described the failure of recent clinical trials of vaccines. They described the difficulties faced in vaccine development, with the need to address a number of components in any vaccination strategy: "Murine models of infection suggest that antibodies, specifically opsonophagocytic killing antibodies (OPK), antitoxin antibodies, and anti-attachment antibodies, combined with T cell immunity, specifically TH17 responses, are needed for broad and potent protection against *P. aeruginosa* infection".

An important factor that has hampered efforts to develop vaccines for human *P. aeruginosa* is the existence of many different serotypes. Many vaccine candidates have shown some effect against their homologous strain, but failed when used against differing serotypes (Hoggarth et al. 2019).

4.6.3 Conclusions

While the early work of Burrell et al. indicated that vaccination against *P. aeruginosa* could reduce the severity of flystrike under some circumstances, a number of factors act against the usefulness of this approach to flystrike control:

- Uncertainty around the absolute need for fleece rot as a pre-requisite for body strike; two aspects –
    - evidence that fleece rot odours are not required to attract female flies to sheep
    - evidence that bacterial growth is not required in order for 1st instar blowfly larvae to establish strikes.
- The proliferation of many different bacteria at fleece rot sites (often in the absence of *P. aeruginosa*).
- The lack of association between fleece rot and breech strike.

An important distinction needs to be made here between:

1) therapeutics for preventing fleece rot (e.g. vaccination against *Pseudomonas sp*.), and
2) breeding for resistance to body strike by selection based on indirect criteria associated with both fleece rot and flystrike (as reviewed in section 4.8 of this report).

The former aims to prevent bacterial growth that occurs as a consequence of extended periods of wetting, but does not address the ability of extended wetting at the skin level to provide conditions for flystrike independent of bacterial growth (as described in section 4.6.2.1). On the other hand, breeding by selection based on body confirmation characters (e.g. shoulder / withers) and wool quality characteristics (e.g. wax / suint ratio) aims to select for sheep with characteristics that impact on the degree of wetting of the skin after rain periods, and will therefore impact on occurrence of both fleece rot and flystrike.

4.7 Scouring

4.7.1 Causes and impact of scouring

Scouring and breech soiling (dags) are recognised as being the major causes of breech strike. Blowflies are attracted to the odour associated with the prolonged wetting of the wool around the breech area from faeces (and urine). Subsequent oviposition at the site leads to the initiation of a strike.

The literature on the causes of scouring, and association with flystrike, was reviewed thoroughly by Jacobson et al. (2019 and 2020). The main factors responsible for scouring, as identified in these review articles, are summarised here:

1) Worm species and regions

- Worm species: principally *Trichostrongylus* spp. (including *T. colubriformis* and *T. vitrinus*) and *Teladorsagia cicumcincta*; *Nematodirus* spp. may be involved in young sheep and lambs; not *Haemonchus contortus* (Barber's Pole Worm), which has a different pathophysiology.
- Most important in southern Australia, with winter rainfall (south-east Australia) or Mediterranean climates (southern Western Australia); scouring outbreaks in winter and early spring when the number of infective larvae on pasture is highest.

2) Direct and indirect effects of worms

- The intestinal-dwelling worms species (*Trichostrongylus* spp.) damage the intestinal surface, including villus atrophy (which leads to a decrease in absorptive capacity) and crypt cell hyperplasia (which leads to increased water and electrolyte secretion).
- The abomasal-dwelling *T. circumcincta* causes stretching and damage to the abomasal gastric pits, leading to a rise in pH and a decline in acid digestion.
- Changes to gut motility, resulting in reduced transit time of digesta (both for intestinal and abomasal-dwelling worm species).
- The host immune response to ingested worm larvae, resulting in increased gut permeability, leading to increased loss of fluid, plasma and mucins.

3) Sheep age and direct / indirect impacts of worms

The relationship between adult worm burden and scouring varies according to sheep age, and is associated with the level of immune response in the host animal:

Young sheep: young sheep show a lower immune response to incoming worms (they have had less time to acquire an effective immune response compared to mature sheep), resulting in higher worm burdens, and scouring.

Lambing ewes: lambing ewes experience a transient decrease in immunity to worms, resulting in higher worm burdens, and scouring.

Mature sheep (> 12 months): scouring is not directly related to adult worm burden in most cases; the host immune response to earlier worm life stages (third and fourth stage larvae) is more important (as described below).

4) Scouring associated with low worm burdens in mature sheep (sometimes referred to as 'hypersensitivity scouring'):

- Associated with a heightened inflammatory response to incoming third and fourth stage larvae.
- Common in mature sheep (they have acquired a higher degree of worm immunity than young sheep); can be triggered in older sheep by exposure to a relatively low number of worm larvae.
- The degree of the response is governed largely by the level of immunity to worms acquired by the animal; therefore, is related to the degree of previous exposure to worms.
- Is heritable: therefore, suitable for genetic selection (further detail given below).
- The inflammatory immune response to incoming larvae is not necessarily protective (won't prevent establishment of the worms) and therefore the degree of the response is not related to adult worm burden; hence selecting sheep based on low FEC ('resistant' animals) will not reduce propensity for scouring.

5) Nutritional scouring:

- Most commonly associated with young lush pastures and rapidly growing forage; these are rich in non-structural carbohydrates (which may lead to subclinical acidosis), plant proteins, and macrominerals, particularly potassium. While suggestions have been made as to the pathways whereby each of these components may lead to scouring, direct evidence is limited.
- Pasture species implicated as possible causes of scouring include cape weed, forage oats, phalaris, and various brassica crops.
- Movement of sheep from pasture to forage crops may lead to scouring, due to lag period in adaption of rumen micro-organisms to a new feed type.
- Alkaloid toxins produced by fungi on perennial ryegrass also linked to scouring in lambs.

6) Bacterial infections:

- Bacterial enteritis and scouring is described as 'an important and emerging disease syndrome, particularly for weaned sheep in the high winter rainfall areas of southern Australia' by Jacobson et al. (2019).
- Two major organisms involved: *Yersinia* and *Campylobacter*.
- During an outbreak, up to 60% of sheep can be affected and the mortality rate can exceed 10% (Slee et al. 1992).
- Distinguished from low worm burden gastrointestinal nematode scouring in mature sheep by its occurrence in younger animals, response to treatment with antibiotics, and lack of response to anthelmintic treatments.
- Stanger et al. (2018) recently showed that *Yersinia spp.* were responsible for scouring outbreaks occurring across 24 farms in south eastern Australia. There were significant levels of resistance to sulphonamide drugs, while treatment with oxytetracycline was effective.

7) Protozoa:

- much less important than worms as a cause of scouring
- primary organism is *Eimeria* (coccidiosis), but can also involve *Crytosporidium* and *Giardia*
- coccidiosis affects very young lambs, especially when ewes are in poor condition; older lambs develop immunity

- chemical control products available, but use not normally warranted

4.7.2 Prevention of scouring

4.7.2.1 Drench and grazing management strategies for control of high worm burden scours

The most direct means to control high worm burden scouring in lambs is through the use of anthelmintics, ideally combined with some awareness of the drug resistance status of worms on the property in order to ensure the use of effective anthelmintic products.

Reducing worm intake from pasture involves the use of grazing management strategies to reduce the numbers of infective larvae that are on pasture at certain times of the year. The WormBoss web site provides advice for graziers in different regions of Australia on the preparation of low worm risk paddocks. For instance, the advice for the Western Australian and South Australian winter rainfall regions consists of (reproduced from the WormBoss site; accessed May 27, 2020):

- Rotational grazing with sheep
  Compared to set-stocking, this typically involves creating a higher stocking rate with larger mobs (at least twice the set-stocking rate) and introducing them to the paddocks when the pasture is about 7 cm high and grazing down to 3 cm high. Aim to have a non-grazing rest period of at least 2 months in winter and 3 weeks during the active pasture growth phase.
- A non-sheep use in at least the 6–8 weeks prior to use with weaners or hoggets
  - Grazing paddocks with cattle
  - Cropping
  - Haymaking
  - New pasture establishment
- Grazing with adult sheep that have a tested low worm egg count (less than 50 epg)
- Grazing with sheep only in the 30 days after they have received an effective drench

4.7.2.2 Breeding for resistance to scouring

There have been a large number of studies on the heritability of dagginess in sheep flocks in Australia and New Zealand. The heritability for faecal consistency has been estimated at around 0.22, although there is considerable variability between studies that have focused on different sheep breeds and sheep of different ages, and across different regions (James 2006; Jacobson et al. 2019, 2020). Heritability of dag score, from studies conducted in Australia and New Zealand, have been as high as 0.60 (Harvey et al. 1984), 0.50 (Watson et al. 1986), 0.54 (Baker et al. 1991), 0.40 (Shaw et al. 1999), 0.41 (Scobie et al. 2008), 0.55 (Greeff and Karlson 2009), 0.31 – 0.37 (Pickering et al. 2012), and 0.33 – 0.44 (Pickering et al. 2013) indicating that genetic selection for reduced scouring is possible.

An Australian Sheep Breeding Value (ASBV) for dag is available through Sheep Genetics Australia. The ABSV is derived from dag scores taken from older animals than used for assessments of breech wrinkle and breech cover (as a degree of prior worm exposure is required for hypersensitivity scouring to occur), and is referred to as Late Dag, and denoted as LDAG ASBV. There are a number of issues that may however impact on the uptake by industry and usefulness of this measure:

- Inconsistency of scouring year-to-year due to interactions of sheep age / previous exposure to worms / larval load on pasture / pasture composition (nutrition).
- The need (for ideal use of the ASBV across a mob of sheep) to allow all sheep time to scour without management intervention once scouring starts to occur in some animals; prior to

crutching; 60 days after the season break following a worm burden (when one exists) or when 30 - 40 % of the flock is scouring.
- The need to assess animals at a later age than for the other flystrike-related traits such as wrinkle and breech cover.

FlyBoss provides guidance for farmers in winter rainfall zones wishing to select based on dag score, with culling of high dag score hoggets in spring recommended.

Importantly, programs focusing on selection for increased resistance to intestinal nematodes (low faecal egg counts) will not reduce the prevalence of scouring (Larsen et al. 1999, Jacobsen et al. 2020). Indeed, there is evidence for an association between worm resistance and an increased propensity for scouring. Hence, it is recommended that, in winter rainfall regions, breeding efforts need to focus on the two traits, with selection for both worm resistance and dag score (Jacobson et al. 2019).

4.7.3 Conclusions

Scouring and dags are recognised as being major causes of breech strike, particularly in southern flocks. Several recent review papers have described the causes and impact of scouring in some detail; we have summarised this information for the present review. It is clear that scouring can result from various factors: high worm burden in young sheep, high worm burden in lambing ewes, low worm burden scouring in mature sheep, feeding on some types of pasture, and some bacterial and protozoan infections. The use of anthelmintics to control worms, as well as grazing management strategies to reduce exposure to worms, can reduce scouring that is due to high worm burdens. Prevention of low worm burden scouring is more complex, and requires an increased understanding of the mechanisms responsible for the phenomenon, and increased knowledge of the dynamics of sheep interactions with worm larvae on pasture in the time period prior to the onset of the scouring.

A number of studies have examined the heritability of dagginess. Overall, the heritability scores have indicated that genetic selection for reduced scouring should is possible. An ASBV for dag score is available, however a number of factors impact on practical aspects of its use in the field. Limitations of this ASBV, and recommendations on how to improve its usefulness, are addressed further in the Breeding sections of this report (4.8 and 6.8).

4.8 Breeding

4.8.1 Introduction

Breeding more resistant sheep was one of the earliest approaches considered to countering the blowfly problem in the early stages of the sheep industry, and current general consensus is that breeding more resistant sheep will be key amongst approaches for controlling flystrike in the future. The main focus of early flystrike was control of breech strike and to a lesser extent body strike, reflecting the relative importance of the two forms of strike (Colvin et al. 2020). There are now a number of estimates for heritability for a range of Merino types which suggest that resistance to both breech strike and body strike is moderately heritable (Raadsma et al. 1991, Mortimer et al. 2009, Smith et al. 2009, Greeff et al. 2014). However, because the incidence of flystrike is often low or intermittent and management is geared to suppress the occurrence of strike, direct selection against strike is often inefficient and, hence, the identification of indirect characters for flystrike is critical to the development of effective selection programs (Atkins and McGuirk 1979). There is an extensive literature reporting studies towards the identification of indirect characters for breech strike, and to a greater extent body strike, including sheep conformational characters, wool and skin traits, immune response, differences in the fleece microbiome, sheep odour and most recently gene markers and genomic indices. The numbers of papers that have been published on various potential indicators of breech and body strike resistance is large and has previously been reviewed (McGuirk and Watts 1983, Raadsma and Rogan 1987, Cottle 1996, Norris et al.2008, Mortimer et al. 2009, Sandeman et al. 2014, James 2006. Greeff et al. 2014, Raadsma et al. 2020) and we will only focus on the key characters here. Breech strike most often occurs in association with urine or faecal staining of the breech wool whereas body strike most often follows fleece rot or dermatophilosis, associated with wetting and bacterial growth in the fleece. At this stage there seems to be little overlap or correlation between susceptibility to the two types of strike, and they need to be considered as separate traits in breeding programs.

4.8.2 Breech strike

The role played by breech and tail folds in susceptibility to breech strike was recognised as a key factor very early in the emergence of flystrike as an important problem in Australia, and it was noted that susceptibility was highly repeatable, with the same sheep likely to be restruck each season (Joint Blowfly Committee 1933). Seddon et al. (1931) divided sheep into three broad susceptibility categories described as relatively insusceptible, moderately susceptible and definitely susceptible on the basis of breech conformation. Seddon and Belschner (1937) later provided a more detailed description of features of the various skin folding patterns in the breech and around the tails of sheep in the three classes and discussed their role in determining susceptibility to strike. The association between this classification and susceptibility to strike was confirmed in a number of other early studies (Seddon et al. (1931), The Joint Blowfly Committee (1933), Mackerras (1936) and Seddon and Belschner (1937).

However with the development of the mules operation in the 1930s and arguments by the proponents about its advantages in comparison with breeding “even one small wrinkle might allow the development of a susceptible area” (Joint Blowfly Committee 1933) momentum in breeding breech strike resistant animals was lost and research became more focussed on improving techniques of mulesing and tail treatment (Morley and Johnstone 1984). It was not until the establishment of the AWI breech strike resource flocks in the winter rainfall (Mediterranean) climate of WA and in NSW in the summer rainfall environment at Armidale that significant further research into breeding for resistance to breech strike resumed (Greeff et al. 2016, Smith et al. 2016). The

studies by Greeff et al. (2009, 2014, 2018), Smith et al. (2009, 2016) have confirmed the overwhelming importance of breech wrinkle in the development of breech strike, both in the Mediterranean climate in WA and in the summer rainfall zone of NSW and have focussed on the implications of and optimal methods for incorporating selection for breech strike resistance in breeding programs.

However, breech wrinkle alone does not account for all the observed variation in breech strike (Greeff et al. 2018). It is well recognised that one of the major effects of mulesing, in addition to removing wrinkles, is increasing the area of bare perineal skin, which renders sheep less liable to urine staining and dags (Johnstone and Graham 1941, Reid and Jones 1976). As a result, as the need to reduce reliance on mulesing grew there was an associated interest in increasing the area of bare perineal skin genetically (Scobie 1997) both by utilising across breed variation (Scobie 1997, Scobie et al. 2002), but also by selecting within populations for extreme phenotypes such as that reported in a South Australian Merino flock and a number of other Merinos flocks since (Edwards et al. 2009). This study showed that the heritability of this bareness trait was moderate to high (0.45 ± 0.02 in lambs, 0.38 ± 0.02 in adults) and there were no significant unfavourable associations with the other economically important traits identified. In addition, Scobie et al. (2002) in New Zealand scored breech bareness from 1 to 5 in mixed breed flocks and showed that those with greater bareness scores were less likely to be struck. Subsequent studies have confirmed that crutch and breech wool coverage and based on Visual Sheep Scores (AWI and MLA 2019) are both heritable and genetically associated with flystrike resistance (Crutch 0.27-0.36 WA, 0.20-.32 NSW) and although the estimates for breech wool were more variable between ages (0.34-0.61 WA) (0.09 to 0.59 NSW).

The role played by urine staining and scouring/dag score in susceptibility to breech strike susceptibility has changed somewhat from the conditions prevailing when the early breech strike research was conducted. Beginning in the 1940's there was increasing use of improved pastures, with resultant higher stocking rates and gastrointestinal parasite problems and an associated increase in the importance of scouring as a predisposing factor for breech strike (Watts et al. 1979). In addition, there was increasing use of radical mulesing, often with a shorter tail, during this period (Morely and Johnstone 1984). The importance of the different management systems on breech strike predisposition was underlined by Watts et al. (1979) from their survey of the flystrike problem in NSW from 1972-74. They noted in that in radically mulesed Merino ewes, scouring was the main predisposing factor, urine stain was minimal and breech strike was negligible where scouring had not occurred. However, in unmulesed ewes, breech strike was invariably associated with urine staining and of 1776 breech strikes in unmulesed ewes, 1624 were scored as 'heavily stained'. A similar increase in the importance of urine stain as a predisposing character for breech strike seems likely as the use of mulesing decreases in current day flocks.

Scouring is now one of the most important predisposing characters for breech strike in the more southerly environments such as SE western Australia where scour worms predominate (Greeff et al. 2018), but less important in areas where *Haemonchus contortus* is the major gastrointestinal parasite. In the southern flocks, wrinkle and dag score were consistently the two main characters associated with the occurrence of breech strike. In the WA resource flock, the genetic correlations between dag score and breech strike were high) 0.48 to 0.82 for early dag score and 0.59 to 0.67 for late dag score and dag score was moderately to highly heritable at both ages (Greeff et al. 2016). The genetic correlation between dag and breech strike was also high in some data sets in the Armidale flock but the authors note that dag is a 'transient trait', that when it does occur it is most often in young sheep, that it can be attributable to any one of several causes and that dag is seldom a problem in adult sheep in the New England environment. They recommend that scouring be used

as an independent culling tool as if sheep have wet dag or severe stain during a fly challenge, they will almost certainly be struck. An important finding in the WA flocks was that for breech wrinkle, as well as for dag and breech cover there was a strong genetic correlation between unmulesed and crutched, unmulesed and uncrutched and mulesed and crutched sheep and that all data for these traits under all of these management systems can be used to estimate breech strike breeding values (Greeff et al. 2019)

In WA, urine stain had a high heritability at weaning and in yearlings (0.55 (0.09) and 0.81 (0.14), respectively, but much lower at marking ($h2$ = 0.27±0.06) and very low ($h2$ = 0.05±0.03) at hogget age (Greeff et al. 2014). The authors suggested that the low value at hogget age was most likely caused by the presence of dags in the uncrutched animals that made it virtually impossible to score urine stain accurately. In the NSW environment, urine stain (scored post weaning) was phenotypically correlated with breech strike (0.18± 0.03) (Smith et al. 2016) and had relatively low heritability (0.22±0.05). However, the genetic correlation was negligible (0.06±0.1) and urine stain was not regarded to be a particularly good indirect criteria for breech strike.

Estimating accurate scores for urine staining is difficult, especially in the presence of scouring and this may be a one of the reasons for the low level of reporting for urine staining and lack of a urine stain ASBV value in MERINOSELECT. There is a need to facilitate easier and more accurate methods of measurement of urine stain and instruction should be added to the Visual Sheep Scores booklet to the effect that urine stain should be scored during low dag/scouring periods.

A wide range of other potential indirect characters for breech strike susceptibility were also assessed in the breech strike resource flock projects (Greeff et al. 2016, Smith et al. 2016) but most provided little advantage over the four characters discussed above. One group of factors that may warrant further investigation are tail-associated characteristics such as tail wrinkle, tail width, and tail bare area length (Greeff et al.2016). These correlations are also present in other data sets including those from uncrutched sheep in Western Australia, reported by Greeff et al. (2014). The width of the tail (measured after hogget shearing) had a strong genetic correlation of +0.66 ± 0.23 with breech strike from birth to hogget shearing, with a moderate heritability of 0.22 ± 0.09.: Tail characteristics were also flagged as potentially important in the early studies of (Joint Blowfly Committee 1933, Seddon and Belschner 1937).

4.8.3 Body strike

Fleece rot, associated with a number of bacterial species (eg. *Pseudomonas* spp.) and 'lumpy wool' (dermatophilosis) caused by *Dermatophilus congolensis*, are considered to be major pre-disposing conditions for body strike. There is some question about the obligate requirement for the presence of fleece rot in order for bodystrike to occur (as described earlier in this report), however it is clear that fleece rot and bodystrike most often occur concurrently. The association of bodystrike with the growth of bacteria following rain wetting was indicated by a number of the early blowfly researchers, while the importance of dermatophilosis as a predisposing factor in field outbreaks of bodystrike wasn't recognised until more recently (Gherardi et al. 1983). Whereas research into breeding for resistance to breech strike ceased for many years due to the widespread focus on mulesing, research into bodystrike and its predisposing causes continued through much of this period. As a result, there is currently much more knowledge of the skin/wool microbiome and its relationship to the aetiology of bodystrike available than for breech strike where there has been an extremely meagre research effort in this area. A detailed discussion of fleece rot and dermatophilosis is provided in Norris et al. (2008). Hayman (1953) reported a that resistance to

fleece rot was an heritable trait, and Atkins and McGuirk (1979) found that the genetic correlation between fleece rot and body strike was close to 1.0, suggesting that in terms of selection, they were functionally the same trait. As outbreaks of bodystrike are somewhat intermittent, that incidence of fleece rot is generally higher than bodystrike and because management is geared to suppress the expression of bodystrike, selecting on the basis of fleece rot was considered likely to provide faster gains in bodystrike resistance than direct selection (McGuirk et al. 1978, Atkins and McQuirk 1979). Estimates of the heritability of fleece rot have been variable with sheep type and environment and have usually been in the low to moderate range (0.13-0.41) (Norris 2008, Mortimer et al. 2009). There are fewer estimates for dermatophilosis, but Lewer et al. (1987) using artificial infection estimated a value of approximately 0.14 whereas Raadsma et al. (1992) estimated a heritability of severity of infection between 0.25 and 0.42 depending on the challenge dose used. These estimates were both obtained under artificial challenge and the relationship of these estimates to heritability estimated under field conditions, where wool factors are likely to play a larger role in susceptibility, is unclear.

A very large number and variety of characters have been investigated as potential indirect criteria for fleece rot and body strike resistance, including conformational characters (e.g. shoulder/withers conformation), wool quality characteristics (e.g. wool colour, coefficient of fibre diameter), wool chemical characteristics (e.g. wax and suint content), various measures of wool 'wettability', structural aspects of the fleece (e.g. staple and tip formation), immune response, both to challenge by *Lucilia* larvae and to fleece rot bacteria. In addition, it is clear that some professional sheep classers are very skilled in identifying more resistant animals (Raadsma et al. 1987). All of these characters have been reviewed elsewhere and most have shown association in some flocks or conditions but not in others (Norris et al. 2008; McGuirk and Watts 1983, Raadsma and Rogan 1987) The two most consistently related to date, although not in all flocks, appear to be unscoured wool colour and fibre diameter variability (James et al. 1987a, b, Raadsma and Wilkinson 1990, Raadsma 1993).

Walkom and Brown (2014) noted that one of the key factors holding back the incorporation of resilience and resistance traits, such as flystrike resistance, into formal breeding indices for breeders is the derivation of accurate economic values. An early start towards this end was made for fleece rot and bodystrike (Ponzoni1983), but further work in this area is needed for bodystrike resistance and more particularly, for breech strike resistance. It is clear both from breeder experience and from the research conducted into resistance to fleece rot and bodystrike that it is possible to make significant genetic gains in improving resistance using well-designed breeding programs. However, from the evidence to date it appears that the key factors determining resistance to breech and body strike are different, and the two will need to be treated as separate traits in any breeding program to increase overall flystrike resistance.

4.8.4 Breeding programs

More recent papers have focussed on rates of improvement in breech strike that can be made by selection on the basis the key breech characters, wrinkle, dag and cover. Richards and Atkins (2010) confirmed that fleece weight will be reduced if single-trait selection for wrinkle is applied, and they noted that if wrinkle is included in a carefully designed selection index, breech wrinkle can be reduced with little associated reduction in rates of gain in production traits. Furthermore they note that the accuracy of selection and rates of gain can be increased by using the correlated characters neck wrinkle and body wrinkle together with breech wrinkle to provide a greater accuracy of assessment, and by using the sheep breeding values available from Sheep Genetics, which also

utilise information on the performance of relatives in estimating breeding values. Brown et al. (2010) estimated the within-flock and across-flock genetic correlations between breech strike indicator traits and production traits recorded on the SheepGenetics data base. Again there were some significant antagonisms between wrinkle score and production traits, most notably fleece weight and fibre diameter. However, they showed that using index selection and depending on the emphasis placed on breech wrinkle in the index, reductions of 0.4 to 0.9 units could be achieved over a 10 year period while maintaining reasonable rates of genetic gain in production traits. They also noted that using across-flock selection for breech wrinkle from MERINOSELECT, together with within-flock selection, in the choice of breeding stock is likely to lead to considerably faster genetic gains than reliance on within flock selection alone. Hatcher and Preston (2017) examined the phenotypic associations of breech wrinkle and breech cover with key production traits, with a view to within-generation selection and management strategies. They found a similar negative association between wrinkle traits and wool production as in the other studies, but a favourable correlation with liveweight and a number of wool quality traits. Breech cover had a similarly favourable correlation with liveweight, but no significant phenotypic associations with other wool production or quality traits. They concluded that Merino producers could implement a range of current generation selection and management strategies to reduce the number of breech strike susceptible animals in the flock with little detrimental impact on key production traits.

Brien et al. (2020) extended this work by including all three of the main indirect criteria for which ASBVs are available in MERINOSELECT and examined the rates of gain in flystrike resistance that could be made by adding flystrike as a trait to three MERINOSELECT indexes (Dual Purpose Plus (DP+), Fibre Production Plus (FP+) and the Merino Production Plus (MP+). They showed that substantial genetic gains in flystrike resistance could be made without unrealistically compromising rates of genetic improvement in the other production traits and present a number of scenarios as examples. They conclude that reduction of breech strike to levels similar to those achieved by mulesing is achievable after 10–20 years of index selection with a relatively minor reduction in rates of gain in other traits.

All of the above-mentioned studies indicate the need for the use of suitable selection indices to minimise the effects of unfavourable correlations with wrinkle to maximise rates of improvement in breech strike resistance, and concurrent rates of gain in production traits. SheepGenetics now provides ASBVs for dags, wrinkle and breech cover that allow breeders to select indirectly for breech strike resistance as well as an ASBV for fleece rot which can be used to assist selection for bodystrike resistance**.** However, there are currently no indexes that include breech strike traits available from Sheep Genetics. Clearly there is an urgent need for the development of breech strike indexes to aid growers attempting to improve breech strike resistance. In addition, there are still relatively few industry records contributing to the estimation of ASBVs for a number of the major flystrike traits, most particularly for difficult to assess traits such as dag score, breech wool cover and urine stain. Collection of more industry data will greatly increase the accuracy of ASBVs for these traits and applicability to different industry breeding objectives and management regimes. There is a need to encourage much more widespread phenotyping for flystrike traits in industry flocks, as well as from research flocks where applicable, for inclusion in the SheepGenetics data base.

Assessing dags and urine stain is not a simple task from a number of perspectives and there is also a need to facilitate practical ‘useability’ of breech strike traits, particularly dags and urine stain for sheep breeders. The possibility of using indirect measures for these traits should be investigated, for example faecal consistency for dag score, and an indication that urine stain be assessed at times of

low scouring/dag in the Visual Sheep Scores document may help increase the accuracy and ease of scoring of urine stain.

The use of image analysis and machine learning, discussed by Raadsma et al. (2020), could significantly facilitate the ease and accuracy of measurement of breech traits and should be investigated. One could easily imagine a camera or cameras strategically mounted in a raceway and positioned for optimal capture of breech images, potentially providing more accurate breech phenotypes and removing a lot of the variability associated with subjective scoring. The possibility of multiple repeat measures to further increase accuracy would also become a relatively trivial undertaking.

One of the largest barriers to selection for breech strike resistance is undesirable correlation with fleece weight and fibre diameter. A number of the papers above make the point that the best way to optimise selection for flystrike resistance and production characters together is inclusion of flystrike as part of a breeding index. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance and this should be considered a longer term priority. However, in the interim, guidelines for breeders for the best use of breech (and other) ASBVs to reduce breech strike susceptibility while maximising gains in other traits could be distilled into a fact sheet available from the Sheep Genetics website.

4.8.5 Genomic breeding values

Genomic selection, whereby the presence of major genes, groups of genes or genomic indices are directly measured to predict genetic merit of breeding stock, is being used with increasing frequency in selection programs for livestock, field crops and horticulture. The potential advantages of using genomic selection for selecting flystrike resistance are substantial as animals would not need to be exposed to strike, or predisposing conditions such as scouring or urine stain for a genetic evaluation to be made. In addition, a genetic value could be attributed to all animals, regardless of whether they are bred in high or low flystrike environments or whether it is a high or low flystrike risk year. Currently research in this area is in its infancy for flystrike-related traits. An initial limited attempt at finding genomic associations for variation in breech strike resistance, based on limited data, found only SNPs of small effect (Dominik, 2019). Additional work, utilising all of the available data from the breech strike selection lines has recently been completed (AWI Project ON-00515), with similar results. However, the report indicated that even though no SNPs of large effect were found, the aggregation of the small effects of many SNPs could be effective in the creation of Genomic Enhanced Breeding Values.

Raadsma et al. (1992) and Engwerda et al. (1996) examined differences in frequency of variants of IgE, TNF alpha, IL1 beta , IL4 and IFN-gamma gene polymorphisms between flocks selected for resistance and susceptibility to fleece rot and flystrike, but found no obvious related differences. Pickering (2013) reported a number of immune, diarrhoea and wool growth genes were associated with flystrike and dag score and Bolormaa et al. (2017) reported on the accuracy of genomic selection for indicator traits related to both breech strike (breech wool cover, crutch cover, dag score, and breech wrinkle) and body strike (fleece rot, fibre diameter variability, and wool colour) in a resource flock of 5726 Merino and Merino crossbred sheep. Although confirmation was provided that all indicator traits were heritable, no genetic correlation with breech strike or body strike susceptibility was reported.

The development of a training population for the estimation of genomic breeding values for fly strike resistance requires the development of a large population of sheep that are phenotyped for fly

strike and genotyped with SNP gene chips. Establishing a purpose-designed flock to accomplish this is expensive and an approach used in other areas has been to integrate data collected from existing genetic evaluation programs and submitted from commercial flocks to form a 'virtual training flock'. This is already underway at some level with the establishment of the MLA genomic resource flock from the previous Sheep CRC Information Nucleus Flock (http://www.sheepgenetics.org.au/Resources/MLA-Resource-Flock); and AWI have committed to contribute over 1500 genotypes from the NSW and WA flystrike resource flocks to this database. The Merino Lifetime Productivity flocks (https://www.wool.com/sheep/genetics/merino-lifetime-productivity/#) Australian Merino Sire Evaluation Association sites and flocks in AWI's 'Improving Resilience in Merino's' project and the 'Genetic Evaluation of Productivity Efficiency and Profit' project represent other potential sources of animals for flystrike phenotyping. In addition, Greeff et al. (2019) proposed progeny testing for flystrike resistance to improve the accuracy of breeding values for elite sires, and suggested that sheep from any such flocks could also provide data for the development of genomic breeding values for breech strike. It should also be possible to use judicious contributions from commercial flocks to add to this data base, as already sought for other traits in the MLA resource flock, without having to institute deliberate challenge testing. Drawing information from a wide range of sources in this way currently seems the most pragmatic way of assembling the large data base required to establish reliable genomic values. It is recommended that an expert panel be assembled to provide oversight of this process and advise on the most efficient and practical way forward (see Breeding recommendations in section 6.8 of this report).

4.9 Trapping

4.9.1 Offal or carcase baited traps

As early as the 1930s Mackerras et al. (1936) showed that high intensity trapping with blowfly traps baited with offal and sodium sulphate could bring about a reduction in flystrike incidence. Using a grid of two types of traps in a series of five experiments on properties near Canberra they achieved an overall reduction in strike of more than 50% in comparison with control properties. Since this time there have been many studies to design new and better traps and improved attractants to improve the effectiveness and practical utility. Anderson et al. (1990) developed a larger scale trapping system based on portable 120 or 240 litre 'wheely bins', painted yellow to enhance attractiveness and baited with a sheep carcase or offal treated with sodium sulphide. These bins had access ports cut in the sides of the bins and treated the interior and the baits with insecticide, and with the wheels attached, were readily moved between sites. Escape of flies through the access ports was prevented by use of toxic insecticide that provided rapid fly kill (most commonly trichlorfon) applied to the offal/carcase bait and inside surfaces of the bin. It was noted by the authors that flies that escaped usually died outside the bin and were rapidly removed by ants.

Although the bait bins collected large numbers of flies, the majority of these were carrion-attracted flies other than *L. cuprina*. Later versions of the bins used a copper mesh covering the access ports with the mesh size designed to increase selectivity of the traps by allowing entry by *L cuprina*, but blocking access of larger blowflies. Although it was claimed that strategic use of the bins at the beginning of the season and located in favoured *Lucilia* habitats, such as along watercourses, near sheep watering points or near sheep yards, reduced flystrike incidence, the veracity of this interpretation was questioned (Cook et al. 1990). However, the bins achieved a degree of adherents and were relatively commonly used by growers, particularly in the more extensive sheep production areas of Australia.

In New Zealand, slightly different designs using 150-L and 200 L offal bait bins with entry ports near the bottom of the bin and an internal cone arrangement to trap flies and prevent fly escape were tested. These traps did not require the use of insecticide. Use of these traps placed at a density of 1 trap/10 ha and baited with fresh sheep offal every 2 weeks resulted in a 95% reduction in the *L. cuprina* population on one property over 3 years (Dymock and Forgie 1995), but in other experiments no reductions in fly strike or blowfly populations were observed (Atkinson and Leathwick 1995). Heath and Leathwick (2001) also describe two trials using 200L bait bins with offal baits. One of short duration gave no reduction in strike whereas the other using bins at a density of 0.2 per ha, with trapping commencing before the start of the fly season and bait bins moved with the sheep gave a marked, though statistically non-significant, reduction in strike of 34% (p=0.11).

One of the criticisms of the methods using carrion or offal baiting was that they were not specific and often trapped much larger numbers of other species of flies than *L. cuprina*. This may actually favour *L. cuprina* by removing competition from other species breeding in carcases.

4.9.2 Other attractants and trapping systems

A number of alternate baits have been developed or tested to enhance the efficacy, specificity and utility of trapping. Morris et al. (1998) compared the efficacy of a mixture of *Proteus mirabilis* and gut mucus, shown to be effective in inducing orientation behaviour and oviposition in *L. cuprina,* with liver sodium sulphide baits in an attempt to find stronger attractants for use in traps. Early in the flystrike season the mucus – *P. mirabilis* baits were as effective as the liver-sodium sulphide

baits, but later in the season the liver baited traps captured significantly higher numbers of *L. cuprina.* In Britain, where *L. sericata* rather than *L. cuprina* is the main flystrike species, Broughan and Wall (2006) used freeze dried liver baits to increase the utility of trapping. In this study pyramidal sticky traps baited with rehydrated freeze dried liver significantly reduced the incidence of flystrike, though not fly numbers. This apparent paradox was explained by the immigration of flies from surrounding areas. Morris (2005) found that a synthetic bait consisting of a sulphur containing volatile compounds encapsulated in a slow release casein matrix caught a higher proportion of gravid *L. sericata* than a liver/sodium sulphide bait, but the attractiveness wore off after 17 days and it was no more effective than the liver bait overall. There was no difference between the two traps for L. cuprina, but only low numbers of this species were trapped. In an interesting variation to traditional trapping, Smith and Wall (1998) tested the effect of cloth targets impregnated with sucrose and 10% triflumuron, a growth regulator insecticide, placed around the periphery of a sheep pasture at approximately five targets per hectare and baited with liver baits. This reduced the density of *L. sericata* to almost zero and fly numbers remained significantly lower than on control farms throughout the period that the impregnated targets remained in the field.

Most notable amongst alternative trapping systems used in Australia have been Lucitraps™ developed by the Queensland Department of Agriculture and Fisheries, and subsequently sold commercially by a number of different companies. Many different attractant preparations were tested in the development of LuciTraps (Urech et al. 1993, 2004), resulting in a final Lucilure™ system with three components butyric acid, 2-mercaptoethanol, indole and 20% sodium sulphide solution (Urech et al. 2004). The trap itself consists of a translucent UV-stabilised plastic bucket and a removable flat, yellow lid with entrance cones that allow sheep blowflies to enter but not leave. These entrance ports are sized to prevent the entry of larger blowfly species that compete with *L. cuprina* in carcases. The lid is attached to the bucket via a twist-and-lock design and brackets are built into the lid to hold the bottles of LuciLure. Once uncapped, wicks in the top of the three bottles (LuciLure A, B and C) emit the attractants into the air for up to six months. Recommendations were to use these traps at a rate of one per hundred sheep. Importantly, no insecticides are used in the trap and the flies usually die from dehydration and starvation. Emptying the trap is usually not required as this is accomplished by ants that rapidly find the traps and remove the dead flies.

Trials consistently demonstrated reduction in *L. cuprina* populations when the LuciTraps were used according to instructions (Urech et al. 1993, 1998, 2004) and studies carried out across 5 Australian States demonstrated an average reduction in mean fly catches from 19.4 to 7.7 flies per trap (Urech et al. 2009). In addition, the Lucitraps were shown to be relatively selective, with *L. cuprina* comprising 59% of trapped flies, *Chrysomya spp*. and *Calliphora spp*. 9.3% and 1.1% of the catches, respectively, with other flies (mainly Sarcophagidae and Muscidae) making up the rest (31%) (Urech et al. 2009). In South Africa, where the main strike species is also *L. cuprina* the ability of Lucitraps to reduce fly populations was also demonstrated (Scholtz et al. 2000). However, in one study there was no reduction in fly numbers. This was thought to be due to an influx of *L. cuprina* from surrounding sheep properties, a potential difficulty that needs to be taken into account when designing a mass trapping campaign to reduce strike incidence.

Although an accompanying reduction in strike incidence was not demonstrated in many of these trials, this was often because of low strike incidence in the control areas (Urech et al. 1993, 2009). However, a comprehensive study conducted in 2003, comprising four separate experiments over 3 years with Lucitraps used at the recommended rate of one trap per 100 sheep, indicated a reduction in flystrike incidence of between 38% and 55% (Ward and Farrell 2003).

These traps have been marketed commercially by a number of companies in Australia, most recently by Bugs for Bugs, Toowoomba, Queensland. However, these traps are not routinely used for flystrike suppression as they are generally considered not cost-effective, due to the large number of traps needed for good effect and maintenance required keep the traps functionally effective.

4.9.3 Push-pull strategy

An alternative approach to attract and kill or localised monitoring, is the 'push-pull' strategy advanced for *L. cuprina* by Rice (1986). This strategy uses odorants or other repellents to repel insects from normally attractive hosts (push) while simultaneously attracting insects to an alternate area or traps, where they can be removed (pull). Push-pull strategies have been used in practice for crop pests, and considered for pests of cattle (Hassanali et al. 2008), but never tested with sheep. The application of repellents (see section 4.2) in parallel with traps could improve the effectiveness of trapping, but with current knowledge it seems unlikely this would be economically viable.

4.9.4 Mass trapping vs strategic trapping

It has been suggested that spatially or temporally strategic trapping may be a more economic option, with limited trapping during low density periods, or in designated areas where blowflies persist (McKenzie and Anderson 1990). In addition, simulation modelling with *L. sericata* in the UK has suggested that, in seasonal environments, early deployment of traps at a time of year when fly densities are low may be the most effective approach to their use (Wall 2012). The notion of a threshold below which the incidence of strike is determined primarily by fly numbers and above which fly abundance is always sufficient and numbers of susceptible sheep is the major strike limiting factor (Mackerras 1936, Monzu 1983; Wardhaugh and Morton 1990) appears to support this proposition. In addition, McKenzie and Anderson (1990) have demonstrated that early season insecticide treatment of sheep prior to *L. cuprina* emergence from overwintering that functionally removes early season breeding sites on sheep for the first generation of flies, can reduce flystrike incidence in comparison with the application of treatments once flystrike risk becomes apparent and trapping of the early emerging flies may have a similar effect. Although this has been suggested to present a more efficient approach to the use of traps, this has not been experimentally validated. However, where trapping is to be used it seems critical that trapping is initiated early in the season, prior to, or at least coincident with, early emergence. In pastoral areas where flies persist through low strike periods in localised foci, these habitats are likely best targeted as a location for traps.

4.9.5 Monitoring fly numbers

In most areas the flystrike season commences once *L. cuprina* begins to emerge from overwintering. Trapping in late winter and regularly checking the traps is an efficient way of determining when the over-wintering blowfly population of sheep blowfly first emerges and can assist in timing sheep treatments or perhaps the implementation of strategies such as early season treatments. In addition, a rapid increase in *Lucilia* numbers can be indicative of the commencement of strike waves. Knowing when sheep blowflies are active, or preferably, anticipating their activity, gives farmers time to plan optimal control strategies. The detection of *L. cuprina* in traps was one of the key parameters in the early warning system for body strike developed in the 1980s (Monzu et al. 1983). Lucitraps are particularly well suited to monitoring because of their specificity for *L. cuprina*, reducing the need to search through large numbers of flies, and their translucent sides making it easy to see when flies are present in the traps.

4.9.6 Conclusions

- *L. cuprina* populations can be reduced by intensive trapping, but the high density of traps required to bring about reductions in strike incidence make this rarely an economic option.
- It has been suggested that temporally and spatially strategic trapping at times when *L. cuprina* densities are low, for example at the commencement of the season when flies are emerging from overwintering or in preferred *L. cuprina* habitats may be the most efficient approach, but this requires confirmation.
- There has been considerable research towards the development of better attractants and trap designs, but it seems unlikely that further investment in this area will result in significant improvements in the efficiency of trapping, or reduction of strike incidence.
- It seems that flytraps will be best used to monitor fly populations, particularly for indicating the emergence of *L. cuprina* from overwintering, and to assist in the design of optimal control programs.

4.10 Forecasting and detection of strikes

4.10.1 Detecting strikes

Flystrike management can be divided to two key elements, the implementation of management procedures to prevent sheep from becoming struck and the timely detection and treatment of strikes when they occur. The labour costs of 'going around the sheep' monitoring for mobs for flystruck sheep, particularly important to maintaining the health and welfare of sheep, amounts to a significant part of flystrike management costs when fully budgeted. If strikes are not detected early sheep develop fever, cease feeding and death may result within six days (Broadmeadow 1984, Guerini 1988). Early detection of flystrike is difficult and continual monitoring of flocks is required to enable timely treatment of struck sheep. In Britain it is a legal requirement to inspect sheep daily for signs of flystrike during high risk periods (National Animal Disease Information Service, https://www.nadis.org.uk/). Accurate methods of predicting strike outbreaks would allow more strategic application of preventative flock treatments if prediction is early enough to enable mustering and treatment of sheep before strikes begin and may reduce pesticide use by avoiding unneeded treatments. Good prediction methods could also enable producers to increase the efficiency of monitoring by increasing the frequency and intensity of inspections during periods of high strike risk.

Flystruck sheep display characteristic behaviours, in particular standing with their head lowered, twitching their tail and trying to bite the affected area (Anderson et al. 1988). As the strike progresses, sheep develop inappetence, don't graze, appear listless and often become separated from the mob. The strikes develop an offensive odour and dark stains often appear on the wool from the presence of serous and larval exudates. However, the strike may be well advanced by the time visual signs are apparent, particularly in the case of body strike. The odour is very characteristic and farm dogs that can pick out struck sheep for treatment are well known in the sheep industry. In addition, Greeff et al. (2013) showed that dogs can be trained to identify sheep that are susceptible to strike even before strikes develop.

In some flocks there is a high incidence of covert strikes. These are small strikes which Wardhaugh and Dallwitz (1984) define as strikes that are only detected by intensive inspection of the sheep. In some instances these are just strikes in the early stages which later develop to much larger strikes. In other cases, particularly in the case of strikes in footrot lesions or pizzle strikes, they tend to persist undetected for some time causing pain to the sheep and potentially providing a mechanism of maintenance for sheep blowfly populations. These strikes are, by definition, difficult to detect and present a problem for early detection of strikes based on visual inspection. Despite the labour costs involved in monitoring flocks for clinical signs of strike, there has been little investigation of alternative approaches to manual inspection.

As early detection of flystrike is difficult and continual flock surveillance is required to enable timely treatment of struck sheep, Cramp et al. (2009) examined the potential of using electronic nose technology to detect struck sheep. The results indicated that the E- nose could accurately distinguish flystrike odour from that of dry wool on days 1, 2 and 3 of strike development in all experiments ($P < 0.05$) and also detected flystrike odour on the day of larval implantation (day 0) in three of four experiments. The study also showed that the E-nose could accurately distinguish strike odours from those of urine- and faeces-stained wool). Furthermore periods of 'sniffing' as short as 2 seconds and sensors placed 0.7m away from the sheep both gave accurate discrimination of strike. The authors note that with the rapid advances currently being made in electronic nose technology, solar power

and communication systems, the vision of remote strike detection technology that can notify managers of the presence of struck sheep in the mob, or even potentially interface with E-sheep technology to draft off struck sheep (Rowe, 2006), seems realistic.

Grant et al. (2020) from examination of video footage of struck and unstruck sheep confirmed that both qualitative and quantitative assessments identified behavioural differences between fly-struck and non-struck sheep. They suggested that remotely assessed behaviour could provide a low input method for identifying animals that require treatment. The authors also indicate the advances that have been made in the development of biosensors to detect behavioural changes in a number of livestock species including pigs (Matthews et al. 2016) and dairy cattle (Rutten et al. 2013) and suggest similar possibilities for the detection of flystrike in sheep.

4.10.2 Forecasting strikes

In order to avoid unnecessary flock treatment for flystrike prevention many growers only treat after strike is detected in their flocks or when weather conditions are suitable for strikes to occur. Other growers treat prophylactically regularly to protect sheep through high risk periods. In both cases the ability to predict when strikes are going to occur can assist in optimising flystrike control programs.

Monzu et al. (1983) describe the development of a prediction system for body strike to assist sheep owners to time jetting before body strikes occurred. The system used a number of cues including the presence of *L. cuprina*, as indicated by trapping at the start of the season, greater than 4mm of rainfall, maximum temperature of greater than 17^{o}C and wind speed less than 30 km per hour (assessed on the Beaufort scale) required for *L. cuprina* activity and fleece remaining moist for at least 24 h to enable egg hatch and larval survival (assessed from a fleece stand). If all of these cues occurred together it was expected that strikes would begin to become evident 3-4 days later if preventative strike treatment was not applied in the interim. Although there was some success with use of this system for detecting body strike outbreaks it was not widely adopted, particularly in areas where breech strikes were frequently a problem.

With a view to the development of better prediction of flystrike and the development of decision support systems to help producers optimise their control options and towards better understanding of the factors that regulate the incidence of flystrike, Wardhaugh and Morton (1990) modelled the incidence of flystrike in the Shoalhaven valley in NSW. They demonstrated that the weekly incidence of flystrike was significantly related to the abundance and activity of gravid flies and various measures of temperature, rainfall and pasture growth. These variates accounted for 76% of the deviance in body strike and 58% of that in breech strike and suggested that it may be able to forecast periods of flystrike with an acceptable level of accuracy on the basis of weather data alone. However the model developed for these analyses took no account of the age or sex of the sheep struck, or of the variable effects of flock management on sheep susceptibility.

In follow up studies (Wardhaugh et al. 2007) large scale flock monitoring programs were instituted in three disparate climatic areas, the Southern Tablelands of NSW near Gunning, the Northern Tablelands near Inverell and on Flinders Island in Bass Strait. The principal objective of this study was to clarify the roles of animal husbandry, weather and fly abundance in determining strike incidence in the different regions and classes of sheep and to assess the extent to which the different regional models could be combined into individual models effective for explaining the incidence of different types of strike in different sheep classes and different production zones.

They found that the base model only required the environmental inputs of daily rainfall, mean daily temperature, and relative humidity at 9.00 am for prediction and did not require any knowledge of fly density or fly activity to provide an acceptable standard of prediction. However, they concluded that because predictions of future weather in Australia were currently limited to a period of less than a week, with those forecasting lead times the models were unlikely to have significant prescriptive value as day-to-day management tools for graziers. An alternative approach to predicting flystrike was taken by Ward (2000) who found that flystrike incidence in Queensland flocks, as estimated from the reported use of flystrike chemicals, was significantly greater in months in which the southern oscillation index (SOI) was positive. He suggested that a useful early warning system could be developed based on the significant correlation between flystrike incidence and the SOI up to 6 months previously with the highest correlation (r=0.33) observed with the SOI 2 months earlier. Whether this association is also apparent in data from areas outside of Queensland, or whether the correlation calculated could provide practically useful levels of prediction of strike does not appear to have been assessed.

Although Wardhaugh et al. (2007) indicated that their model was of limited practical use for forecasting strike, they noted that the models could have significant value for strategic planning and for developing decision support systems for growers based on historical climate data, for example aiding growers to optimise times for implementing fly control practices such as crutching, shearing and strategic chemical applications. The model has now been used in the development of the decision support Tools in the FlyBoss Flystrike Risk Simulator which estimates the risk of flystrike in a particular geographical location and then makes adjustments for management options such as shearing and crutching, breech modification, timing of chemical treatments and the effect of breeding in reducing susceptibility to strike (Horton and Hogan 2010). Two different tool formats are presented; “Optimise Treatment Tool” and “Compare management Tool” http://www.flyboss.com.au/sheep-goats/tools/flystrike-quick-tools-online.php). As noted by Wardhaugh et al. (2007) use of these base models is only limited by the quality of the climatic data available. One significant improvement made in the Flyboss models is the more accurate localisation of predictions based on the Silo climate data base (https://www.longpaddock.qld.gov.au/silo/). Whereas initially these models relied on climate data from the nearest BOM weather station for prediction, through the Silo data base weather data can now be interpolated in 5 km spatial grids providing superior local accuracy for users. The FlyBoss models, using simulations utilising the model of Wardhaugh et al. (2007) and incorporating local weather data and sheep susceptibility factors can assist producers who wish to optimise their current flystrike control programs, or perhaps modify their programs to reduce reliance on mulesing or to take account of the potential development of resistance to some chemicals on their property. The models have also been used for other purposes for example to compare the likely cost and management implications of moving from a mulesed to unmulesed flock in different environments (Lucas and Horton 2013), to assess the relative costs and management implications of the use of a fixed annual treatment date or waiting until a fixed proportion of the flock is struck in different environments (Percival and Horton 2014) and to assess the financial and management implications of using a strategic early treatment approach to the application of preventative chemicals (Horton 2015).

4.10.3 Conclusions

Welfare and economic imperatives will increasingly demand more effective and labour efficient methods of monitoring sheep for flystrike and early detection of flystrike will be critical. The extensive production systems of Australia and the tendency for struck sheep to become separated from the main mob will be problematic for photographic and e-nose methods, although

appropriately designed e-noses appear to be able to detect strikes very early in their development. Sensors attached to the sheep, able to detect characteristic behaviours or physiological changes associated with strike and able to signal remotely would seem likely to provide a better option and are worthy of investigation.

Australian flystrike models have demonstrated their ability to inform decision making in flystrike management, and have been utilised to provide interactive decision support modules for sheep owners through ParaBoss. However, their utility in predicting strike over practically useful periods is currently limited by short reliable weather forecasting timelines. Whether the use of the SOI can assist earlier forecasting of strike periods seems worthy of further investigation. The ongoing use of available models in decision support for wool producers and future applications for research in this area should be considered on the basis of the specific use anticipated.

4.11 Genetic Manipulation of the Fly Population

4.11.1 Past efforts in blowfly genetic blowfly control

This section discusses genetic manipulation or sterilisation of *L. cuprina* flies rather than breeding more resistant sheep. These methods seek to bring about suppression or eradication of the pest population by the release of flies of the same species that have been modified to confer sterility or cause genetic death in the target pest population. These methods are also known as autocidal control and are usually used in area-wide strategies focussed on eradicating pest populations. With *L. cuprina* this is likely to be regional eradication or suppression, although in the most well known instance where the sterile insect technique (SIT) was used to eradicate the New World Screwworm in the Americas, eradication was from multiple countries (Krafsur 1998).

The sterile insect technique uses mass releases of male insects that have been irradiated using gamma radiation to cause damage to insect chromosomes or sperm, effectively rendering them sterile. With many species of flies, including *L. cuprina*, the females only mate once. Therefore, if a female mates with a sterile male she is functionally sterilised for life. With serial mass releases of sterilised males the chance of a fertile female finding a fertile mate is reduced to close to zero and a population can be eradicated from the release area. In its most well-known use, noted above the SIT method has been successfully used to eradicate New World Screwworm flies, which has biology not unlike *L. cuprina*, from North and Central America (Krafsur, 1998). This method has also been used for eradicating regional incursions of insects, such as fruit flies in fruit fly-free areas of Australia and an incursion of screwworm flies in Libya and of tsetse flies from the Island of Unguja in the Zanzibar archipelago (Vreysen et al. 2000). The enormous potential cost-benefit rewards that can accrue from successful use of this approach is illustrated by the New World screwworm program in the Americas where it has been calculated that the direct benefits realised each year from the program are equal to or greater than the total cost of the sterile male release program over the fifty years of its operation (Vreysen and Robinson 2011).

Utilisation of this approach requires the establishment of significant infrastructure ('factories') to rear the large numbers of flies required for successful use of this approach, development of a release strategy (usually from aircraft) and desirably, technology to separate or incapacitate the female flies. However, because of the widespread areas in which *L. cuprina* is found in Australia and the lack of suitable geographic or climatic barriers similar to those present in North and Central America this approach has generally been considered uneconomic for widespread use in Australia. Regional use of sterile male may be viable for *L. cuprina* control in some situations, and has previously been considered for use in Australia but would require a significant research and development effort to establish and is unlikely to be economically tenable unless a production facility can service multiple areas or a large population of at-risk sheep (King et al. 1992).

To address the barriers to the use of SIT in Australia, in the 1970s, CSIRO investigated the use of compound chromosome strains, sex-linked translocation strains and female killing systems to suppress or eliminate *L. cuprina* populations. These methods rely on the release of flies containing a mutation that they would pass on to at least some of their offspring, and which over time, bring about elimination of the population. Following the examination of a number of approaches, the CSIRO program focussed on the use of a 'field female killing' (FFK) strain of the blowfly (Foster et al. 1993). Females of this strain were homozygous for an eye colour mutation (resulting in white eyes and functional blindness) that is lethal to the females once they are released into the field. The male flies are not blind, however they are semi-sterile and mate with wild type females in the field to pass on the mutation. Fly populations are suppressed in two ways; firstly, only a small proportion

(approximately 50 %) of the eggs hatch, and secondly, the males pass on their mutation to all surviving daughters, causing the elimination of a proportion of their descendants (through blindness), and hence, a gradual reduction in the fly population over time.

A trial using this strain on Flinders Island (land area 36 km^2), off South Australia, in 1985-1986 was successful in suppressing the blowfly population to undetectable levels. The last release of flies was in Autumn 1986. In the absence of further releases, flies were again detected at low levels in the spring and summer of 1986, and the population had recovered by Autumn 1987. The recovery of the blowfly populations was suggested to be due to flies immigrating or inadvertently re-introduced from the mainland.

A subsequent trial was conducted on the Furneaux islands in Bass Strait (main land mass Flinders Island, area = 1,367 km^2). The trial failed for a number of reasons, including: practical difficulties with the mass rearing of flies, the unstable nature of the mutations, and the reduced fitness of the released flies compared to the wild type field population. Further work with these strains was eventually not pursued because of the operational difficulties and funding constraints.

4.11.2 Recent technological advances for producing flies suitable for a genetic control program

Huge advances in molecular biology techniques, the recent availability of the sheep blowfly genome (Anstead et al. 2015, 2016) and the development 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) (Lacroix et al. 2012) or potentially using gene drives to spread deleterious (often sex-linked or stage specific genes) through fly populations. Notably the RIDL system, which has been used for control in mosquitoes is very similar in principle to the female killing strain developed earlier by CSIRO, but whereas the CSIRO strain used recessive mutations to confer lethality, in the RIDL system, the RIDL males carry a dominant female-lethal gene. Modelling in other insects suggests that releasing fertile RIDL males would be more efficient for population suppression than SIT as the female offspring would die and the male offspring, which don't strike sheep, would survive to pass on the female lethal genes to the next generation, thereby theoretically reducing the number of flies that need to be released.

Research is currently underway, funded by AWI, to identify critical genes in *L. cuprina* and may facilitate the design of genetically modified strains suitable for use in area wide autocidal approaches. In addition significant advances with *L. cuprina* have been made in this area in the laboratory of Dr Max Scott at North Carolina State University. Recent work has focused in part on the use of molecular biology techniques to develop insect strains that carry repressible female lethal systems. This allows for the normal functioning of female flies for reproduction within the mass rearing facilities, however, the female flies carry genes that are lethal under certain environmental conditions that can be manipulated in the rearing facility. Hence, when the specific environmental conditions are provided, the female flies die, leaving just the males for release into the environment. This significantly reduces the costs or production of flies for release and reduces the likelihood of inadvertent release of fertile female flies. Matings between these fertile male flies and wild type females in the field would produce either female offspring that would die, or male offspring who could then pass on the female lethal genes to the next generation.

Heinrich and Scott (2000) described such a system for the vinegar fly, *Drosophila melanogaster*. A gene associated with cell death (leading to death of a whole organism), was placed under the control of a genetic element that was inactive in the presence of the antibiotic tetracycline, but active in its

absence. This element was in turn placed under the control of a female-specific genetic element. In females, when tetracycline was present, the cell death gene was inactive. When the tetracycline was absent, the cell death gene was activated, leading to death of the flies. Males were unaffected (as the initial control element was female-specific) and hence could develop normally in the presence or absence of the antibiotic. Hence, flies could be bred on medium supplemented with the antibiotic over a number of generations to build up the population size, and then a tetracycline-free diet could be used for the generation prior to release into the field, leading to the death of the female flies at the late larval / pupal stages, leaving a population of only male adult flies for mass release.

This technology was then successfully applied to establishing blowfly strains with a tetracycline-repressible female lethal genetic system (Heinrich et al. 2002; Concha et al. 2011, Li et al. 2014). Concha et al. (2011) showed that a tetracycline-repressible system could be incorporated into the blowfly alongside a fluorescent marker gene. The female flies died as expected at the late larval / pupal stages (in the absence of tetracycline). However, the fluorescence intensity of early first instar female larvae was much greater than male larvae. Hence, a fluorescence-based cell sorter could be used to separate the females and males at this early larval life stage, rather than wait for the females to die at the late larval / pupal stages. An ability to remove females at the earlier life stage from the generation to be released into the field would result in significantly reduced costs for providing feed for larvae in an insect rearing facility. It was however noted by Li et al. (2014) that the capacity of the cell sorter would need to be matched with the high-throughput requirements of the rearing facility.

More recently, the issue of the timing of the death or removal of female flies from the generation that would be released into the field has been further addressed. Scott and colleagues have added embryonic-specific elements into the female-lethal tetracycline system (Yan and Scott 2015, Yan et al. 2020). This results in the death of females at the very early embryonic stages of the fly's life cycle when tetracycline is absent, compared to their death at the late larval or pupal stages in the earlier strains. Hence, in insect rearing facilities, the females would be removed from the field-release population before the need for any feeding. In addition, after fly release, the female offspring of matings between the field females and released males would die before they could cause any damage to the animal. In the earlier system that was not under embryonic-specific control, such female offspring would have been able to develop as normal on the sheep before dying at the late larval or pupal stage. However, importantly in terms of flystrike occurrence, in this embryonic-female-killing system, and in the earlier larval killing system, the male offspring of matings between field females and released males would establish strikes as normal on sheep in the field.

It is clear that the advances in molecular manipulations of the blowfly described by Scott and colleagues have provided opportunities to generate flies showing characteristics that may be suitable for genetic control programs based on the release of fertile male flies carrying female-lethal genetic systems. However, it should be noted that the transgenic female-lethal lines were made from a North American *L. cuprina*, and crossing with Australian *L. cuprina* strains would be required to enable evaluation of the ability of the strains to suppress field populations of Australian sheep blowflies.

4.11.3 Gene drives

Gene drives are an immensely powerful tool that can allow targeted genes to preferentially spread through a population (Gould 2008). Gene drives have been identified in nature e.g. Homing endonuclease genes (HEG) in bacteria, and these bacterial genes have been introduced into mosquitoes, but the advent of CRISPR and associated technologies have now enabled the design of purpose-driven gene drives to target critical genes in pest populations (McGraw and O'Neill). HEGs

have already been introduced into a number of mosquito species and modelling has predicted that they would be able to eliminate populations within a few years after introduction (Deredec et al. 2011). Clearly the use of gene drives would enable the design of genetic control strategies that could help overcome the logistic and cost the barriers presented by the large areas over which *L. cuprina* is found in Australia. The recent cloning and ongoing characterisation of the sheep blowfly genome would aid the identification of critical and specific *Lucilia* genes that could be targeted in such an approach.

The development of artificial gene drives relies on the use of CRISPR together with appropriate RNA sequences to alter or silence a specific gene, or insert a new one. Once a gene drive is engineered into an animal's genome, the insect's progeny will inherit the drive on one chromosome and a normal gene from its other parent. During early development, the CRISPR portion of the drive cuts the other copy. The cut is then repaired using the drive as a template, leaving the progeny with two copies of the modification, rather than one. In this way, the change is passed on to up to 100% of offspring, rather than around 50% and in this way the gene is 'driven' into the population.

An example of an engineered gene drives is a drive introduced to mosquitoes that targets an essential fertility gene called *doublesex*. In cage experiments using mosquitoes with designed drive in place the female mosquitoes could not bite and did not lay eggs and within 8–12 generations, the caged populations produced no eggs at all (Kyrou et al. 2018). Clearly use of such a technique presents immense possibilities for use against a pest such as *L. cuprina*, where other genetic controls are rendered infeasible by the extensive areas over which *L. cuprina* is dispersed in Australia and the extremely low densities at which it exists.

However, gene drives, once introduced to a population can spread by themselves and there are serious concerns about the unpredictability of gene drives (Deardon et al. 2008, Scudellari 2019). For example, the drive could spread beyond the targeted population with unwanted consequences, or mutations or other undesirable genes could be spread along with the targeted genes. Gene drives have the potential to alter entire ecosystems and to change the course of things in ways that cannot be predicted. It has been suggested that they could, in theory, negatively affect human health by causing a parasite or pathogen to evolve to be more virulent or to be carried by another host (Scudellari 2019). For this reason it is considered that the use of gene drives is extremely risky, likely to be subject to substantial societal concerns and is unlikely to be approved for field use by regulators, at least in the short term. However, it should be noted that because of the enormous benefits possible from the use of gene drives methods to potentially override or otherwise counter or reverse them are already under development and significant research programs to this end are currently in place (Gould et al. 2008; Scudellari et al. 2019). A full consideration of gene drives is beyond the scope of this review. However, for further information on the issues surrounding the potential use of gene drives, the advantages and disadvantages and recommendations for the way forward see the article by Deardon et al. (2008), 'The potential for the use of gene drives for pest control in New Zealand: a perspective'.

We recommend that this is not an area that MLA should currently invest in, but that a watching brief be taken on this area of research, in particular developments in the technology, societal opinion and regulatory aspects of the use of gene drives towards a reconsideration of investment in this area in the longer term

4.11.4 Feasibility

The success of the sterile male approach in eradicating New World screwworm fly from north and central America was partially due to the particular geography of this area, and that New World Screwworm died out of through most of north America in winter. There was only a small area in the eastern USA, in southern Florida where NWS survived the winter, and this was initially targeted for eradication. In the western areas of USA reinvasion occurred from Mexico and Central America each summer, facilitating a sterile male approach in this area. In contrast, the enormous areas in Australia in which *L cuprina* persists with few natural geographic or climatic barriers presents a significant hurdle for an area wide genetic approach. A possible exception is in Tasmania, or other geographically-bounded areas such as Western Australia (Pauley), or other Islands such as King Island or Kangaroo Island in SA where there is substantial sheep production.

An extensive economic evaluation of the economic feasibility of CSIROs autocidal techniques for the genetic control of the sheep blowfly in Australia (focussing on the FFK strain) towards use of the strain was carried out by King et al. (1992) in Tasmania. Four different analyses were conducted, for eradication on King Island, for eradication from Tasmania, for eradication from Western Australia, and for eradication from Australia. For King Island the expected avoidable cost incurred by farmers was estimated as $0.55 per sheep per year in 1991 Australian dollars. The major avoidable costs incurred were for jetting and the major losses were sheep deaths. Two factors were found to have major impact on the financial rate of return, the length of time that the area remained free of the pest and the capital cost involved in constructing and equipping the rearing facility. For King Island considered alone, the technique would not be economically viable if the full cost of construction and operating the production facility was to be carried by the project. To be economic it had to be part of a program for a larger area, covering more sheep, or would need to be subsidised.

For the whole of Tasmania, the cost benefit was more favourable. The mean cost per head of flystrike Tasmania-wide was estimated as $0.83. For the whole of Tasmania scenario, the two most important variables were the capital costs and the time needed for eradication. In this case the proposition, including construction of a facility for the whole of Tasmania, appeared sounder and by the third year of the program it was estimated that the benefits from the program should exceed the operating costs. An issue flagged was that many sheep in the State do not incur the assumed average loss, but something significantly less, and this could raise equity issues surrounding collection of levies to support the program. For WA the cost of flystrike per head was estimated as 78.4c per head in the agricultural regions and 88.5c per sheep for the pastoral regions. However, the economics of the program in WA were not as good as in Tasmania, largely because of the ongoing costs to prevent reinfestation, but also because it would take an extra 6 years to achieve eradication using a facility constructed at the minimum feasible cost.

When Australia was considered as a whole the cost benefit of the approach was reasonably good for the area in eastern Australia taking into account the high rainfall zone and sheep wheat belt from if fly production can reach its forecast potential, but extending the eradication areas to include the low sheep density pastoral zone diluted the return on investment. This did not take into account the costs of ongoing maintenance of barrier zone between the pastoral zone and the more easterly sheep production zones. However, it was considered that maintaining an ongoing production facility between the eradication and non-eradication zones was not prohibitive as the ongoing costs are well into the future and small relative to the benefits. Overall, it was concluded that the return to investment looked quite favourable for larger, higher sheep density areas if problems associated with the large scale rearing of the FKK strain evident at the time of the study could be overcome.

However, the analysis suggested that delays to the eradication schedules, which they indicate experience has shown are probable, has a major impact on the financial return possible.

In addition, the numbers of flystrike susceptible sheep included in the eradication area have a major effect on cost benefit and current sheep numbers in Australia are very much lower than the 170 million sheep population at the time of the survey. The proportion of Merinos, which are more of a strike risk than meat producing breeds, is also much lower than in 1991.

Clearly, current costs of production and sheep and wool prices are very much different to those at the time of this analysis and recent technological developments that affect costs of rearing and distributing flies could have a major effect, and clearly a re-assessment of the economic feasibilility would be required before embarking on any autocidal approach. In addition, implicit in the assessment made by these authors is that such an approach would be funded with a grower levy. The political will and likely grower response to imposition of such a levy would be a key consideration, and precedents suggest that funding with a voluntary levy is unlikely to be tenable.

The use of autocidal controls is considered a high risk / high reward option. Eradication, either regional or national could lead to enormous benefits, both in terms of production costs, labour saving and welfare impacts. However, implementation of such an approach entails high cost for the development of production infrastructure, colony maintenance and rearing expense and the costs of distribution and release. Nothwithstanding, the development of male only production methods will reduce both production costs, and depending on the genetic modifications being used, a reduction in the number of flies required for release in comparison to that required for a sterile male approach. Systems based on gene drives are much more likely to provide an economic option, but are also much more likely to be prevented by regulation and societal concerns and their future use will depend on the development and acceptance of methods to address concerns about unanticipated deleterious outcomes.

4.11.5 Conclusions

Whereas most current sheep blowfly control programs rely on direct animal treatments through methods such as crutching, mulesing and the application of insecticides, area wide genetic approaches aim to directly target blowfly populations. This could potentially provide significant labour savings and welfare benefits and enhance Australia's reputation as a producer of safe and ethically produced sheep meat and wool. However, the large areas, over which *L. cuprina* is found in Australia, usually without geographic barriers, pose significant difficulties for any genetic control method, such as the sterile insect technique (SIT), which relies on the production and release of high ratios of modified:wild flies.

Genetically modified strains such as compound chromosome strains and field female killing strains (FFK) have shown promise for use in area wide control programs and can substantially reduce the production costs and release ratios in comparison with conventional SIT methods. A two-component female embryo lethal system for *L. cuprina* has been developed by Max Scott and colleagues at North Carolina State University. This system could be suitable for use in a genetic control program, but would need to be tested for compatibility with Australian strains of *L. cuprina*.

The recent availability of the sheep blowfly genome will significantly aid the identification of genes that could be utilised in genetic control programs. Approaches to the area-wide control of *L. cuprina* based on gene drives or perhaps *Wolbachia* (see section 4.3.5.3) which could drive deleterious genes through a blowfly population and require the production and release of much lower numbers of flies than sterile male or like methods may offer a possible genetic approach in the longer term once

effective safeguard counter measures are developed. However, whether they can be used in future will be determined largely by societal factors and regulatory bodies. A watching brief should be taken on this technology.

Area wide genetic control is a high cost, but high potential benefit approach to flystrike control. A preliminary cost benefit analysis should accompany any future project proposals for area wide genetic approaches.

5. Conclusion

5.1 Key findings

The present study has highlighted a number of areas that warrant attention in order to provide long-term solutions to the issue of flystrike. The areas recommended for attention fall into two categories according to whether they are at a stage requiring substantial research input, or whether they are at a stage where a significant level of knowledge already exists, such that the emphasis now should be on greater adoption by the industry.

Research areas identified include:

- drug resistance management (drug resistance diagnostics, modelling of drug-use strategies)
- chemical and biological therapeutics
- novel delivery methods for chemical and biological agents
- development of more readily-measurable breeding indices for flystrike-related traits, including genomic breeding values
- prevention of nematode-induced scouring.

Areas where advances can be made in flystrike control through the greater adoption of well-recognised management approaches include:

- optimal drug-use practices (resistance management strategies)
- guidelines for breeders on how to best use current flystrike-related ASBVs
- management practices (including breeding and optimal anthelmintic use) to prevent scouring.

We have described the position of the sheep ecto-parasite drug market in the commercial priorities of animal health companies as an important determinant of progress in delivering new therapeutics for flystrike control. This relationship means that it is important to coordinate research activities into novel therapeutics with the companies. We suggest that the outcome of research presently underway on flystrike vaccination should direct further investment in this area, with appropriate consideration to be given to the biological factors that make such a strategy difficult to achieve. We have highlighted areas where the availability of the blowfly genome could potentially provide new impetus to developing intervention strategies, including in the areas of drug-resistance diagnostics, new chemicals, vaccination, and genetic manipulation of blowfly populations. However, we also highlight the fact that commercial and feasibility considerations will act to temper the potential for the genome to act as the basis for providing practical control options in some of these areas.

5.2 Benefits to industry

The project has provided several recommendations (shown in detail in section 6, below) that describe research and adoption pathways that will be important for the sheep industry to develop sustainable and long-term flystrike controls. We have highlighted research into drug resistance, novel therapeutics, improved breeding indices for flystrike-related traits, and the causes of scouring, that could potentially have a significant impact on flystrike control. We also recommend that increased emphasis be placed on adoption of drug-use patterns that are known to reduce the rate at

which resistance develops, as well as increased adoption of current know-how and management practices that could have an impact on the susceptibility of sheep to flystrike and scouring. Progress in these areas will be important for ensuring that the red meat industry is able to deal effectively with the issue of flystrike in coming years.

6. Future research and recommendations

Here we provide recommendations for future research and adoption activities to provide long-term control of flystrike:

6.1 Insecticide discovery and resistance / blowfly genome

Given the current reliance of the industry on chemicals, the barriers for developing new insecticides for flystrike control, and the time that it will take to implement changes to the sheep industry to reduce the reliance on chemicals, we suggest that increased efforts are required to preserve the usefulness of the currently-available chemicals for as long as possible. This effort should be applied to minimising the impact of drug resistance on the efficacy of the current chemicals. Components of this should focus on delaying the emergence of resistance, slowing the spread of any resistance that has already emerged, and minimising the severity of any resistance (that is, to minimise the impact of resistance on flystrike protection periods). While resistance may be inevitable in the long term, delaying its impact until longer-term strategies (such as breeding) can have a greater influence on the industry is vital.

We suggest that attention should be paid to:

1) encouraging drug rotation strategies

   It is important that the need for insecticide rotations in flystrike control programs is communicated effectively, and implemented. The information provided by FlyBoss on the various chemical classes, and the products within each class, should be an important component of guiding product choice on farms.

2) exploring the use of drug combinations to delay resistance

   Modelling studies should be used to examine the merits of using combination products in order to delay resistance in the blowfly.

3) the use of diagnostics to inform graziers on the resistance status of fly populations on their property / in their region

   We suggest that testing of drug sensitivity of field-collected blowfly populations should be implemented on a wider scale than is presently the case. This will provide information on the levels of resistance that currently exist to each of the major chemical classes, and therefore allow for informed drug-use decisions to be made, that is, to avoid the use of chemicals to which resistance is already present. We suggest that the resistance monitoring service offered by NSW DPI (Narelle Sales) be supported in order to allow the continued provision of information on drug sensitivity in fly populations recovered from sheep by graziers in regions across Australia. Increased scale of this service may be warranted, with some level of industry-wide coordination.

   Serious consideration should be given to developing a rapid resistance test for the blowfly. Any rapid resistance test would need to utilise molecular or biochemical methods that could

be applied to the fly specimens submitted to the laboratory, rather than waiting for two generations of breeding as required for the current resistance test. Research would be required to define the molecular basis of resistances shown by, or likely to emerge in, the sheep blowfly to cyromazine / dicyclanil, ivermectin, spinosad, and imidacloprid. The study of cyromazine / dicyclanil resistance could utilise the resistant fly populations described in Sales et al. (2020). Such research would also utilise knowledge on resistance mechanisms used by the sheep blowfly to previously-used chemical classes (particularly organophosphates and benzoyl phenyl ureas), as well as knowledge on mechanisms of resistance to cyromazine / dicyclanil, ivermectin and imidacloprid in other insect species. The sheep blowfly genome will be an important resource for the development of such molecular-based insecticide resistance diagnostics.

6.2 Repellents

1) Research laboratories are likely to continue efforts to develop controlled release matrices for use with volatile repellents for protection of humans against mosquitos. Developments in this field may provide leads for such an approach to livestock parasite control. With a view to future options for blowfly control, it would be useful to monitor technological advances in controlled release technologies.
2) A re-examination of the potential of GH74 for blowfly control will depend on commercial considerations within animal health companies. An important aspect of GH74 will be the usefulness of the compound in markets beyond just sheep blowfly control. Engagement with animal health companies, with a view to supporting the development of contact-based repellency approaches in general, would be warranted.

6.3 Biological control

1) There are a number of issues associated with biological controls that will act against their development for blowfly control, including stability, cost of production, and low efficacy compared to currently-used chemicals. Research organisations will likely continue to identify biological agents with insecticidal activity. Some of these may show activity against the sheep blowfly. We suggest that attention be paid to these research efforts in order to identify any that may have application to blowfly control. Engagement with the companies where possible on development of biologicals for blowfly control is warranted.
2) We identify several research gaps that impact on the use of biological controls:
    - given the substantial benefits from an area wide approach to control, the potential use of *Wolbachia* warrants further investigation.
    - Information on the spatial distribution of larvae and pupae in the soil or of temporal and spatial the ecology of soil pathogens to assess whether targeting the soil stages could provide a significant effect on fly numbers, and hence, strike incidence.
3) Given the widespread use of *Bt* as a biopesticide across many areas of insect control, and evidence that it can provide some protection on sheep (from Heath et al. 2004), we suggest that its use against blowfly warrants further discussion / investigation. Efforts to optimise formulation chemistries for its application to sheep in order to maintain the *Bt* endotoxin near the skin at strike sites is a principal area of interest (from Heath et al. 2004), and may also have implications for chemical treatments. However, as mentioned in the insecticide

section of this review, development of any *Bt* product may be hampered by the insect Order specificity of individual *Bt* endotoxins, thereby precluding the use of specific *Bt*-based products across multiple species of livestock or companion animal pests (flies, ticks, fleas, lice), and hence limiting the market size (and hence, company interest in) any product based on a Dipteran-specific *Bt* endotoxin. We note that the use of a *Bt* extract as a biopesticide rather than its use as a live genetically-modified bacterium engineered to carry *Bt* toxins and grow in the fleece, would avoid the regulatory and marketing barriers associated with the genetically-modified technology.

6.4 Novel delivery of flystrike prophylactics and therapeutics

We suggest that opportunities exist in a number of areas for novel delivery technologies to provide significant advances in the use of therapeutics for flystrike control, including the following:

- Significantly increased periods of protection provided by chemical and biological/botanical actives.
- Broadened suite of chemical or biological actives suitable for practical use, thereby increasing resistance management options.
- Chemical release profile that reduces pressure for the selection of resistance.
- Can facilitate the development of genetic approaches such as RNAi for practical pest control by protecting against environmental degradation.
- Many of the developments in nanotechnology are currently occurring in academia, medical research and in areas not related to pest or parasite control. Given the relatively small size of the blowfly strike control market MLA may need to partner in this area.
- Most nano-formulations can be delivered through conventional application equipment.
- Systemic application of flystrike insecticides, particularly when delivered strategically from controlled-release systems, may be a more efficient method of application than topical application as it delivers insecticide to all body sites where strike may occur and removes the potential for 'patchy application' with topically applied treatments. However, systemic delivery increases the possibility for tissue residues and will be applicable to only a very limited suite of actives.

6.5 Vaccination against flystrike

Australian Wool Innovation is currently funding flystrike vaccine projects at CSIRO and the University of Melbourne. This work aims to use the blowfly genome and transcriptome, alongside time course experiments with blowfly larvae, to identify blowfly proteins that play important roles in the establishment of strikes. Such proteins may be candidate antigens for vaccination experiments. The work is also re-investigating some of the antigen classes studied in the 1990s. Recombinant proteins are being produced in protein expression systems and administered to sheep. The effectiveness of these candidate antigens in elucidating serum-antibody-based effects on larval growth is being assessed using *in vitro* assays.

We suggest that the outcome of the current AWI-funded work should guide decisions on further research work in this area. Further investment decisions should consider the level of evidence provided to show that serum from vaccinated animals can inhibit the development of blowfly larvae *in vitro* to such an extent that the ability of the larvae to establish infections *in vivo* would also likely be significantly inhibited. Even with this evidence of *in vitro* effects, there would still be significant hurdles associated with the biology of the blowfly / sheep interaction to overcome before a vaccine could be considered a realistic possibility.

Any discussions of research funding in this area should involve an animal health company as their level of interest, and willingness to invest in the development of a vaccine, are crucial considerations.

6.6 Vaccination against fleece rot

While the early work of Burrell et al. indicated that vaccination against *P. aeruginosa* could reduce the severity of flystrike under some circumstances, uncertainty around the importance of fleece rot as a pre-requisite for body strike, the proliferation of many different bacteria at fleece rot sites (often in the absence of *P. aeruginosa*), and the lack of association between fleece rot and breech strike, suggests that this method of flystrike control would be of only limited usefulness. Hence, we make no recommendation for further work in this area.

6.7 Scouring

We identify three areas with potential to impact on the ability to prevent scouring, and hence reduce the occurrence of breech flystrike:

1) Breeding to reduce the propensity for scouring in mature animals

Scouring leading to breech soiling (dag) is one of the major predisposing factors for breech strike in southern production areas. Breeding for reduced scouring by use of selection based on dag score (LDAG ASBV), provides the dual benefit of reduced scouring and stained wool and reduced breech strike susceptibility. However, current ASBVs are derived from a relatively narrow flock base. Greater recording of dag score in commercial flocks and submission to SheepGenetics should be encouraged (see section 6.8).

2) Research on the basis of low worm burden nematode-induced scouring

It is clear that this type of scouring is important in mature sheep. However, many aspects of its nature remain unknown, and the need for investigation of a number of factors were highlighted by Jacobson et al. (2019, 2020), including –

- The mechanism by which larval intake induces scouring; this may allow the prediction of its likely occurrence, provide a more precise basis for genetic section compared to simple dag scores, and form the basis of diagnostics for use in disease management.
- The dynamics of the interaction of sheep with larvae on pasture; for example, i) the period and extent of worm exposure that is required before the scouring response is triggered; ii) the balance between the time period of exposure to worms prior to Summer in a Mediterranean environment (as a result of month of birth), and the likelihood of scouring in hoggets in the next Autumn due to either high worm burden (lack of immunity in animals born late the previous year), or low worm burden (due to greater exposure to worms in animals born early in the previous year).

- The basis for the sporadic nature of outbreaks among different flocks on a property, and between different farms in a district.
- Occurrence across different breeds of sheep (particularly in meat breeds).

3) An emphasis on worm management practices for prevention of scours

- Larval load on pasture: preparation of low worm risk paddocks, with potential impact on both high and low worm burden scours.
- Effective use of anthelmintics for worm control to prevent high worm burden scours. Worm treatment in response to elevated worm egg counts, or observed scouring in lambs, requires the use of an effective anthelmintic, that is, a drench product to which the worms on the specific property are not resistant. We recommend the promotion of resistance testing using *DrenchCheck* (single product) or *DrenchTest* (faecal egg count reduction test; multiple products) in order to determine which drenches remain most effective on individual properties.

6.8 Breeding

1) More extensive collection of phenotypic data from industry flocks

There are still relatively few industry records contributing to the estimation of ASBVs for some of the major flystrike traits, such as dag score, breech wool cover and urine stain. Collection of more industry data will increase the accuracy of ASBVs for these traits and applicability to different industry breeding objectives and management regimes. Ways of encouraging more widespread phenotyping of breech characters and submission to SheepGenetics should be explored.

Assessment of the main breech traits is labour intensive, difficult and, in the case of urine stain in particular, probably frequently inaccurate. The recording of alternative, more readily measured indirect measures for the main breech 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 be considered. A directive in the Visual Sheep Scores booklet to score urine stain at times of low dag/scouring may help increase the accuracy of assessment of this trait. The potential for use of image analysis of the breech traits, together with machine learning, to improve the ease, accuracy, and standardisation of recording should be investigated.

2) Development of breeding indices and economic values for flystrike

There is a need to facilitate practical 'useability' of breech strike traits in MERINOSELECT for sheep breeders who wish to improve breech strike resistance. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. This will require the development of an economic value (s) for breech strike.

In the interim, guidelines for breeders on how to best use breech-related ASBVs available from Sheep Genetics to reduce breech strike susceptibility while maximising gains in other traits should be distilled into a fact sheet available from the Sheep Genetics website

3) Genomic breeding values and indices

The use of genomic selection is rapidly becoming more widespread in breeding programs for livestock, field crops and horticulture. Genomic values could be attributed to all animals in all years and all environments, regardless of level of flystrike challenge. Establishment of genomic breeding

values requires a very large data base of sheep phenotyped for flystrike resistance and genotyped with a SNP chip. The establishment of a 'virtual' genomic resource flock, based around that MLA Genomic Resource flocks, with data drawn from a wide range of research and commercial flocks towards the development of genomic enhanced breeding values has previously been suggested, and this approach is supported.

We recommend the establishment of a Genomics Implementation Working Group to oversee planning and progress in this area and to determine the most efficient and effective path forward with regard to available resources/resource constraints.

6.9 Trapping

Blowfly traps can be useful for determining the presence of sheep blowflies, particularly after emergence from overwintering, and can reduce flystrike incidence when used strategically, or at high densities. Many different attractants have been tested and improved designs and more easily used bait formulations have been developed, but the range of attraction, critical to the economics of trapping remains a challenge for their use in extensive Australian production systems. We see further research in this area as currently low priority.

6.10 Forecasting and detection of strikes

1) Welfare and economic imperatives will increasingly demand more effective and labour efficient methods of monitoring sheep for flystrike and early detection of flystrike will be critical. The development of flystrike sensing systems, perhaps based on remote or on-sheep behaviour or physiological monitoring requires further investigation and could have both welfare and labour efficiency benefits. Sensors attached to the sheep, able to detect characteristic behaviours or physiological changes associated with strike and able to signal remotely would seem likely to provide a better option and are worthy of investigation.

2) Australian flystrike models have demonstrated their ability to inform decision making in flystrike management and have been utilised to provide interactive decision support modules for sheep owners through ParaBoss. However, their utility in predicting strike over practically useful periods is currently limited by the short time window of reliable weather forecasting. Whether the use of the Southern Oscillation Index can assist earlier forecasting of strike risk periods may be worthy of further investigation. The further adaptation of available flystrike models to extend or develop new flystrike decision support modules for wool producers is encouraged and advances in weather forecasting should be monitored with a view to earlier prediction of strike risk periods.

6.11 Genetic manipulation of the fly population

1) The large areas over which *L. cuprina* is found in Australia pose significant difficulties for genetic control methods such as the sterile insect technique which rely on the production and release of high ratios of modified:wild flies. A two-component female embryo lethal system for *L. cuprina* developed overseas (Max Scott) which could reduce the production costs and release ratios may be suitable for use in Australia, and should be evaluated for potential use.

2) Approaches to the area-wide control of *L. cuprina* based on gene drives or perhaps *Wolbachia* (see section 4.3.5.3) which could drive deleterious genes through a blowfly population and require the production and release of much lower numbers of flies than sterile male or like methods may offer a genetic approach in the longer term. We recommend that this is not an area that MLA should currently invest in, but that a watching brief be taken on this area of research, in particular developments in the technology, societal opinion and regulatory aspects of the use of gene drives towards a reconsideration of investment in this area in the longer term.

3) Area wide genetic control is a high cost, but high potential benefit approach to flystrike control. A preliminary cost benefit analysis should accompany any future project proposals for area wide genetic approaches.

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