

## INVITED REVIEW

# Control of sheep flystrike: what's been tried in the past and where to from here

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Flystrike remains a serious financial and animal welfare issue for the sheep industry in Australia despite many years of research into control methods. The present paper provides an extensive review of past research on flystrike, and highlights areas that hold promise for providing long-term control options. We describe areas where the application of modern scientific advances may provide increased impetus to some novel, as well as some previously explored, control methods. We provide recommendations for research activities: insecticide resistance management, novel delivery methods for therapeutics, improved breeding indices for flystrike-related traits, mechanism of nematode-induced scouring in mature animals. 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 Australian Sheep Breeding Values, and management practices to prevent scouring in young sheep. 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 factors that will impact on the ongoing availability of insecticides for flystrike control and on the feasibility of vaccination. We also describe areas where the blowfly genome may be useful in providing impetus to some flystrike control strategies, such as area-wide approaches that seek to directly suppress or eradicate sheep 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 some control options.

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**F**lystrike is a serious financial and animal welfare issue for the sheep industry. Lane et al.<sup>1</sup> estimated that flystrike costs the Australian sheep industry approximately \$M175 per annum, with more than half of this due to production losses associated with loss of wool growth and value and bodyweight loss. The remainder

of the cost was associated with treatment and prevention, chiefly insecticide use. Breech strike, the most common type of strike in most years, occurs following urine staining or fouling of the breech with faeces, usually when sheep have diarrhoea (scouring). Body strike occurs most commonly over the shoulders or along the backline, usually after the development of fleece rot or dermatophilosis. Body strike often becomes the predominant type of strike in wet years and during fly waves (sudden increases in fly numbers in response to a combination of favourable environmental conditions). Pizzle or belly strike is most common with wethers or rams when the belly wool becomes stained and dampened by urine. Poll or head strike occurs mainly in horned rams and can reach relatively high prevalences in some ram flocks.<sup>2</sup> The Australian sheep blowfly, *Lucilia cuprina*, initiates the vast majority of strikes in Australian sheep.<sup>3</sup> Strikes may also be initiated by *L. sericata* and a number of species of brown blowflies in the genus *Calliphora*, particularly in cooler areas. *Chrysomya* spp., mainly *C. rufifacies*, may also be involved in strikes in a secondary, although potentially very damaging role, once the primary fly species have already established strikes.

A great deal of scientific research has been conducted over many years with the aim of controlling the sheep blowfly. This has significantly enhanced our understanding of sheep blowfly biology, the causes of strike and the factors underlying susceptibility in sheep and has led to a range of advances in control methods. However, new enduring solutions to the problem of flystrike are clearly required. In addition, mulesing, one of the keystone methods of control over many years, is no longer a tenable method of control. 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 long-term control.

This review paper aimed to examine a number of 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. These strategies have included the direct targeting of the larval or adult stages of the blowfly using chemical or biological agents, manipulation of the host immune response, husbandry approaches to reduce the cues that lead to a strike, breeding of sheep less susceptible to flystrike, improved forecasting and detection of strikes, and eradication or suppression of blowfly populations using genetic manipulation techniques. There have been many significant scientific advances over the last few years, for

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example, in various aspects of molecular biology, gene editing, nano-scale technology and remote sensing that can perhaps be utilised to find new solutions 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. We examined past research efforts on different flystrike controls, with a view to assessing whether these may offer promise for long-term control and looked at whether modern technological advances may provide new impetus to these previously proposed control strategies. We also looked at whether completely new research pathways are now possible in the modern scientific environment. 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.

### Insecticides

Control of sheep flystrike has relied on the application of insecticides for many years and, in turn, the sheep blowfly has shown an ability to develop resistance to some of these insecticides.<sup>4,5</sup> This resistance has led to some compound classes no longer being effective as prophylactic treatments (organophosphates and benzoyl phenyl ureas). A recent review of current flystrike control practices in the Australian sheep industry found the most commonly used chemical was dicyclanil (40% of producers) followed by cyromazine (24%), ivermectin (12%) and spinosad (12%), indicating a high industry reliance on two structurally related compounds, dicyclanil and cyromazine.<sup>6</sup> However, recent studies have indicated that resistance to these two compounds is emerging in field blowfly populations. Cyromazine was used widely in the sheep industry for over 30 years before Levot<sup>7</sup> reported that larvae recovered from a property in southern NSW showed resistance to this compound, and to dicyclanil to a lesser extent, in *in vitro* assays. Baker et al.<sup>8</sup> 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. This indicated that the low level of *in vitro* resistance was not impacting the period of protection if the chemical was applied correctly. The original cyromazine-resistant strain was exposed to selection pressure with cyromazine over 13 generations in the laboratory, resulting in a population showing 3.5-fold higher levels of resistance compared to the original field-collected strain.<sup>9</sup> Larval implant trials with this selected strain showed that protection periods were reduced to much less than the label claims: from 14 to <8 weeks for cyromazine and from 18 to 24 weeks to <11 weeks for dicyclanil.<sup>10</sup> 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 periods on sheep if it increased to higher levels over time through further intensive use of the drug in the field.

Sales<sup>11</sup> recently provided an update of *in vitro* insecticide resistance levels towards all of the currently used insecticides in blowflies submitted by graziers from regions across Australia. All flocks tested

from NSW (n = 55) showed resistance to both cyromazine and dicyclanil, while 10 out of 11 from Victoria were resistant to cyromazine and 9 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. Sales<sup>11</sup> and Sales et al.<sup>12</sup> also reported on an *in vivo* trial with strains composed of 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 larval implant trial: strikes were observed (i.e., 'protection failures' occurred) at weeks 3, 4 and 9 for dicyclanil products with protection period claims of up to 11, 24 and 29 weeks, respectively. However, as with the earlier study of Levot et al.,<sup>10</sup> this recent trial used two strains of blowflies that had been exposed to laboratory selection pressure with dicyclanil prior to their use in implants. Hence, the study provides a clear illustration of the potential impact of insecticide resistance on protection periods, rather than actual protection periods likely to be obtained against blowfly populations as they currently exist in the field. Nevertheless, the study highlights the potential impact of drug resistance on the ability of dicyclanil to provide extended periods of protection against flystrike and is likely to raise concerns in the sheep industry. While alternative chemicals are available (ivermectin, imidacloprid, spinosad and cypermethrin), none of these provide the length of protection of some of the dicyclanil-based products (up to 12–14 weeks compared to up to 29 weeks). A further concern is that resistance to all of the other drug groups used for blowfly control has arisen in other insect species or ticks: ivermectin,<sup>13</sup> imidacloprid,<sup>14</sup> spinosad<sup>15</sup> and synthetic pyrethroids (SPs).<sup>16</sup> Hence, resistance in the sheep blowfly to these chemicals may be expected to also emerge in the field in the future.

Bringing new chemicals to the blowfly control market has been in the hands of the major chemical companies as they bear the very considerable costs associated with this process. Most of the currently used chemicals were developed within the companies for the control of pests other than blowflies and then were subsequently developed for use in blowfly control. 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. Dicyclanil also shows this limited spectrum of activity. However, the priorities of the animal health companies have now shifted to such an extent that the development of compounds with such a limited spectrum of activity would no longer occur at any animal health company involved in the discovery and marketing of new insecticides. The priority parasites for these companies are heartworm and fleas in the companion animal market (dogs and cats), and cattle tick, gastrointestinal worms and horn fly/buffalo fly in the livestock market. The sheep blowfly is seen as a low priority by major companies. However, 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. 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. The sequencing of the blowfly genome revealed 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.<sup>17</sup> However, it is unlikely that such information will be utilised directly to develop new flystrike therapeutics given the secondary position of this market in animal health company priorities. It remains possible though that the genome may be useful in identifying a new potential insecticide target that could prompt drug development focusing on a company priority pest. As described above, a secondary outcome may be a flystrike control product in the longer term.

Recent work on the identification of new chemicals for blowfly control provides 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.<sup>18–20</sup> These studies reported on the potent activity of inhibitors of histone deacetylase enzymes against larval life stages of the blowfly *in vitro* and *in vivo*, hence highlighting their potential as insecticides. However, the research to date has focused solely on activity against the blowfly, with the worth of these enzymes as drug targets in other pests of animals not yet demonstrated, and hence 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.

Given the crucial role that insecticides play in flystrike control, and the time it will take for alternative control measures to have a significant impact in reducing the reliance on chemicals (principally breeding, as described below), it is important to preserve the usefulness of the current set of flystrike chemicals for as long as possible. 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.

Methods to reduce the rate at which resistance emerges and to minimise its impact once it is present 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. An important aspect of this is the use of insecticide rotations to ensure that selection pressure is not imposed on the pest population by repeated use of chemicals from the same chemical class, or the use of insecticide combinations.<sup>21,22</sup> The FlyBoss website (<http://www.flyboss.com.au/sheep-goats/>) provides advice to graziers on rotation strategies for chemical use. 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 that are likely to determine the most efficient insecticide-use strategy for flystrike control, for example, the presence and nature of refugia, the mode of inheritance of resistance to the combination components, the stage of the insect being targeted, the site being

treated on the animal, cross-resistances to the component chemicals, prevalence of insecticide-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 on the best insecticide-use strategy for managing resistance in the sheep blowfly.

It is recognised 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.<sup>23</sup> The New South Wales Department of Primary Industries (EMAI, Camden, NSW) has measured drug sensitivity in fly populations in regions across Australia for many years. Information on susceptibility to each of the major chemical classes is provided to graziers who have submitted blowfly samples to the laboratory, thereby allowing for informed drug-use decisions to be made and to avoid the use of chemicals to which resistance is already present.<sup>11</sup> However, the work does have a 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 grazer (6–9 weeks). This time period is a fly biology issue as the laboratory needs to breed the flies for two generations to establish a sufficiently large fly population to allow for the testing procedure to be performed. This 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. Despite this, the resistance information is valuable in being able to direct the choice of early-season prophylactic treatment in the following season. We suggest that an increased scale of this service is warranted, with some level of industry-wide coordination. We also suggest that there is a need to develop rapid molecular-based resistance tests for the blowfly, with a turn-around time of a few days to a week. 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. 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. The sheep blowfly genome will be an important resource for the development of such molecular-based insecticide resistance diagnostics, as will knowledge on mechanisms of resistance reported previously in the sheep blowfly and other insects.<sup>13,14,24</sup>

### Repellents

The historical use of repellents against wool and wound myiasis flies and nasal bot flies in sheep was reviewed by James.<sup>25</sup> Such repellents act to prevent female flies from depositing eggs onto sheep, rather than acting as insecticides to kill blowfly larvae. Repellents are generally much less toxic than insecticides and hence the issues associated with environmental contamination are not as severe.

Vapour-based repellents act through the exposure of adult blowflies to chemical vapours, deterring them from landing on the sheep. However, the volatile nature of such compounds means that they evaporate quickly and thus have very limited residual action.<sup>26,27</sup>

Short-term vapour-based repellents play a small role in current blowfly control as a component of some flystrike 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, while the organophosphate compounds that are also contained in these products act to kill blowfly larvae. The only viable option for the use of such volatiles to provide prolonged protection against the sheep blowfly would be to use a slow-release formulation (discussed below). An exception to this short-term effect was observed in laboratory-based experiments by Callander and James<sup>28</sup> who showed that oviposition on wool treated with tea tree oil (from *Melaleuca alternifolia*) was suppressed for 44 days. It was suggested that persistence may have been due to the extended release of vapours from tea tree oil dissolved in the lipid coating of wool fibres in the sheep fleece. Importantly though, as noted by the authors, 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.' Yim et al.<sup>29</sup> reported on the use of  $\beta$ -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 (CR) formulations.

Contact-based repellents act to deter the fly from laying eggs after it has landed on the sheep and made contact with the chemical agent in the wool of the animal. Work conducted by George Holan at CSIRO in the 1960s aimed to modify the dichlorodiphenyltrichloroethane (DDT) molecule in order to reduce its mammalian toxicity and increase its biodegradability while retaining insecticidal activity.<sup>30,31</sup> 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). Several studies conducted in the early 1980s showed that treatment of sheep with GH74 resulted in significant suppression of oviposition.<sup>32,33</sup> In one experiment, 378 full egg masses were laid on control sheep compared to 15 on treated sheep over a period of 41 weeks. However, the compound was not as effective around the breech of scouring mulesed sheep. Observations of placement of eggs suggested that flies on the treated animals had most likely stood on the wool-free area of the breech to lay their eggs and hence avoided direct contact with the GH74 present on the wool fibres. This limitation may be less of an issue today as more producers switch to a non-mulesing operation, which provides less bare skin on which blowflies can stand to oviposit and avoid contact with treated wool. On the other hand, selection of animals based on the low breech cover in breeding programmes 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. Despite significant promise for the control of various Dipteran pests, GH74 was not commercialised, due to financial concerns in the early 1970s, as described by Ward<sup>34</sup>: '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).'

While SPs are used widely as insecticides for killing insect pests, most SPs also inhibit oviposition.<sup>35</sup> 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, for example, Sectar®, containing cypermethrin and diazinon. 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,<sup>36</sup> and hence, at the concentration applied to sheep, the compounds would act as both oviposition suppressants and larvicides.<sup>36,37</sup> Sales et al.<sup>36</sup> showed that the widespread use of SPs against lice before their introduction for fly control had not resulted in any resistance to the oviposition suppression activity. However, it is not known if this situation still exists today. There are clear advantages in using products that have distinct modes of action against blowflies in order to delay the development of resistance. The oviposition suppression activity of SPs has a further benefit in targeting the adult life stage of the blowfly, in contrast to other current flystrike control chemicals that target the larval stages. The lack of a breech strike claim for the presently available product is however a significant disincentive for its use. Another 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, (<http://www.liceboss.com.au/sheep-goats/>) 'SPs must not be used, even off-shears' to satisfy European Eco-label wool residue requirements.

### Biological control

Biological controls are generally considered to be more environmentally friendly, less prone to residues and safer for the operator than traditional chemical insecticides. In addition, agricultural goods produced in low-chemical and organic systems generally have more favourable consumer acceptance, sometimes accompanied by price premiums. 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 nonchemical approaches to the management of flystrike.<sup>5,38</sup> Despite this, to date no natural biocontrols that appear to exert significant regulating influences on sheep blowfly populations have been identified. Various options have been considered in each of the three general categories of biological control:

- 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
- inundative biocontrol: large numbers of a living organism are applied to flood the pest populations as a biological pesticide. 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

- the use of nonliving biologically derived toxins, for example, plant-derived compounds

The population dynamics and ecology of *L. cuprina* would seem to present significant difficulties for any classical biological control agent to exert meaningful levels of control. *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 fly numbers 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 would likely need to be funded by industry organisations or government.

Most recent research towards a biological control agent for sheep blowflies has focussed on the potential for inundative approaches in which bacteria, fungi, nematodes or potentially entomopathogenic viruses, 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 being 'clean, green and sustainable' and hence have favourable consumer perception. Although registration is still required for bacterial-, fungal- and virus-based formulations, the registration pathway is usually somewhat simpler and considerably cheaper than for chemical agents. 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 compared to chemical pesticides, they can be subject to variability in efficacy, generally have a shorter shelf life, are more prone to breakdown under environmental influences, and can have difficulties with quality control. The use of genetically modified pathogens faces regulatory and marketing barriers.

In the early 1900s, prior to the development of chemicals for fly strike control, significant research was conducted into the possibility of using parasites and predators in classical biological control.<sup>39</sup> Attempts to utilise a naturally occurring pupal parasite *Mormoniella* (*Nasonia*) *vitripennis*, a parasite of *L. sericata* in Europe (*Alysia manducator*), and a predatory beetle, were unsuccessful due largely to factors such as slow breeding by the control agent, unsuitability for harsh environmental conditions, lack of specificity for *L. cuprina* and lower than expected ability to kill blowfly pupae.<sup>40–42</sup> Examination of blowfly larvae collected from flystrikes on sheep in New Zealand identified three species of parasitic wasps; however, the overall parasitism rate was very low (1.1%) and unlikely to contribute in any significant measure to the regulation of blowfly populations.<sup>43</sup>

A number of species of entomopathogenic fungi have also been examined as potential flystrike biological control agents, including *Octosporea muscaedomestica*, *Metarhizium anisopliae*, *Beauveria bassiana*, *B. pseudobassiana*, *Akanthomyces muscarius*, and *Tolyposcladium cylindrosporium*.<sup>44–48</sup> However, a number of factors were considered to act against their usefulness for flystrike control, including, the length of time to kill or impair fertility in adult flies meant that significant oviposition could still occur before death, transmission is likely to be slow between highly dispersed adult flies, and there are doubts over the suitability of the fleece environment for the growth of some pathogens.

Entomopathogenic nematodes (ENs) have also been investigated for control of *L. cuprina*. ENs kill their host through the release of mutualist bacteria which then proliferate in the dead insect. 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. *L. cuprina* prepupae in the soil were shown to be quite susceptible to ENs, possibly due to their relative inactivity, and hence it has been suggested that they could be used to increase mortality in the overwintering soil stages of *L. cuprina*, particularly through introduction into sheep camps.<sup>49</sup> However, the preference of most ENs for a cool and humid microenvironment to achieve high levels of infection, and persist through prolonged periods when few host larvae are present, may preclude their use against the soil stages of *L. cuprina* in many Australian situations.

Given the widespread use of the soil bacterium *Bacillus thuringiensis* (*Bt*) as a biopesticide across many areas of insect control globally, including the control of important pests of broadacre and horticultural crops, as well as nuisance flies and some Dipteran vectors of human disease,<sup>50,51</sup> there has also been interest in its use for flystrike control, with the emphasis on inundative rather than inoculative biocontrol. A number of field-isolated strains of *Bt* have been shown to produce toxins that are active against blowfly larvae *in vitro* and *in vivo*.<sup>52–55</sup> Heath et al.<sup>53</sup> showed that *Bt* extracts applied to patches on sheep were 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, but, 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 a separate large field trial, sheep were treated with *Bt* solution along the backline, and around the rump, and then exposed to natural flystrike. The *Bt*-treated animals showed 36% fewer strikes than control animals, but the difference between control and *Bt*-treated animals was not statistically significant.

*Wolbachia* is a genus of intracellular, maternally transmitted bacteria that can infect a range of arthropod species and filarial nematodes and is considered to have significant potential for control of insect pests and insect-vector-borne disease.<sup>56,57</sup> *Wolbachia* are somewhat different from 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 that are most commonly used in biocontrol. *Wolbachia* infection has a number of different 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. When CI occurs, matings between infected males and noninfected females, or between males and females infected with different strains of *Wolbachia*, produce infertile eggs. However, matings of both infected males and noninfected males with infected females are viable and produce fertile, infected eggs, hence facilitating the spread of the bacterium through the population. CI can be used for direct suppression of insect populations in a similar manner to the sterile insect technique (SIT; discussed below) in a method known as the incompatible insect technique.<sup>58</sup> Fitness effects of *Wolbachia* include reduced insect lifespan, decreased egg viability, reduced pupal emergence and reduced mobility and feeding efficiency.<sup>59</sup> These effects can have a significant impact on survival and reproduction in insect populations and could potentially be used to collapse sheep blowfly populations.<sup>60</sup> While *Wolbachia* has been detected in *L. cuprina* collections from a number of sites around Australia (Perry pers. com.), its biological effects in *L. cuprina* are yet to be characterised and hence its potential for use in the control of sheep blowfly populations remains to be clarified.

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,<sup>28,61–63</sup> plant extracts<sup>64,65</sup> and alkaloids.<sup>66</sup> However, relatively high concentrations of the extracts or oils were required to reduce larval survival significantly in these studies and hence they are unlikely to be useful flystrike control agents, other than as repellents in the case of some essential oils, as described above.<sup>28</sup>

There has been a great deal of interest in the foliar application of double-stranded RNA (dsRNA) molecules for the control of insect pests of crops through the ‘silencing’ (RNA interference) of critical genes in the target insect.<sup>67,68</sup> However, work to date indicates that ingestion of dsRNA molecules by *L. cuprina* larvae is not effective in preventing their development (A. Kotze, unpublished data). A likely explanation lies in the degradation of the dsRNA molecules by blowfly nuclease enzymes in the saliva secreted onto their food material and 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. However, further work to identify optimal encapsulation and release technologies to protect ingested dsRNA from the blowfly nuclease enzymes may be warranted.

#### Novel delivery of flystrike therapeutics

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. 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. The use of such CR technologies has the potential to provide extended periods of protection against flystrike. Whereas traditional formulations of pesticide

depend for prolonged action on a single initial high concentration treatment so that control is maintained until concentrations decay below effective levels, CR systems aim to release pesticides in steady amounts at active levels or to release only at times of infestation risk. Initial doses need not be as large, thereby reducing the risk of tissue residues, environmental contamination, operator exposure and other off-target effects. There has been interest in the use of such technology for sheep parasites since at least the mid-1980s: polymer matrix tags impregnated with cypermethrin against head flies (*Hydrotaea irritans*) in the United Kingdom,<sup>69</sup> insecticide tags against sheep ked (*Melophagus ovinus* L.),<sup>70</sup> tags containing cypermethrin against *Bovicola ovis*<sup>71,72</sup> and diazinon tags against *L. cuprina* poll strike.<sup>73</sup>

CR capsules containing ivermectin and cyromazine have shown potential for ectoparasite control in sheep.<sup>74–77</sup> Studies on the effect of ivermectin rumen capsules designed for use in worm control showed that the capsules provided extended protection against breech strike (strikes reduced by 86%); however, only moderate protection was provided against body strike (27%).<sup>76</sup> The superior performance against breech strike was attributed to the excretion of ivermectin in the faeces of scouring sheep. It was suggested by the authors that strikes in urine-stained areas of the breech may have been responsible for the incomplete protection against the breech strike. It is notable that these results were obtained using a capsule designed for the control of gastrointestinal parasites and it appears likely that capsules purpose-designed for control of flystrike, which 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, designed to provide flystrike-active concentrations of cyromazine in the serum, was shown to provide up to 12 weeks protection from flystrike.<sup>74</sup> An added attraction of this capsule was that the release of cyromazine gave an approximately ‘square wave’ profile plasma concentration, providing very steep decay tails. Thus, the intensity of selection for resistance to the chemical would be lower compared with that from the usual topical applications where extended decay tails are usually observed and are problematic in terms of resistance development. 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. Importantly though, such systemic delivery increases the possibility for tissue residues and will therefore be applicable to only a limited suite of actives.

In recent years, there has been increasing interest in the area of nanotechnology for medical, veterinary and agricultural applications, with approximately 60,000 papers published in the literature since the early 1990s, and the number of patents involving nanoparticles in these areas increasing from near zero in 1990 to close to 40,000 in 2020.<sup>78</sup> For veterinary applications alone, nanoparticle formulations can be divided into polymeric nanoparticles, liposome nanoparticles, micellar nanoparticles, dendrimer nanoparticles and metallo-nanoparticles.<sup>79</sup> 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 nanoparticles are completely inert or biodegradable, with high safety profiles.

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 CR. 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. A number of studies with crop pests have demonstrated enhanced performance in terms of release profiles and photostability of nanoformulations containing insecticides that are known to be active against Diptera, for example, imidacloprid,<sup>80</sup> methomyl<sup>81</sup> and emamectin benzoate.<sup>82</sup> 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 a chemical insecticide against photodegradation and rainfall leaching and provided increased periods of protection against blowfly larvae in comparison to conventional formulations (James et al. unpublished data).

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, extended protection by nanoparticles loaded with garlic oil against red flour beetle,<sup>83</sup> protection of the water-soluble botanical pesticide rotenone from photodegradation by formulation in chitosan nanoparticles,<sup>84</sup> and prolonged action of dsRNA molecules in protecting plants from viral infection when formulated in clay nanosheets.<sup>85</sup>

### Vaccination against flystrike

A great deal of work to develop a vaccine for flystrike was undertaken by CSIRO and the University of Melbourne from the late 1980s until the early 2000s<sup>86–95</sup>, however, the research programme ended with no vaccine being commercialised. The University of Melbourne group focused on antigens that were recognised by the sheep immune system during natural blowfly infestations, whereas the CSIRO group focused mostly on gut-associated ("hidden") antigens that were 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 was assessed using *in vitro* assays in which larvae were fed on serum from vaccinated or control animals, and also, in some studies, with *in vivo* measurements of larval growth or protection against flystrike. A number of trials showed that serum from vaccinated animals was able to inhibit the growth of larvae *in vitro*.<sup>87–95</sup> However, the effect on larval growth was only temporary. Larval growth was inhibited at early time points (usually 20 h) but then the larvae continued to grow and pupate. Hence, such growth inhibition was of little biological significance in terms of preventing flystrike as the larvae would still 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<sup>96</sup> stated that larval growth at 20 h in *in vitro* experiments needed to be inhibited by more than 80% in order to prevent the larvae subsequently developing fully to the pupal stage. Most of the studies conducted in the 1990s reported larval inhibition at much less than this 80% level. An exception was the study by Tellam and Eiseman<sup>93</sup> that reported *in vitro* inhibition of larval growth of >80% at 20 h, alongside mortality of 35%; however, the study did not continue to monitor larval weight gain or mortality at later time points.

The required threshold of larval growth inhibition becomes even greater when translating *in vitro* experiments to an *in vivo* infection as Eisemann et al.<sup>97</sup> showed that larvae at infection sites on sheep ingested 66% less antibody than larvae feeding in *in vitro* assays. In most cases, the effects on the growth of larvae on sheep (*in vivo*) in the CSIRO and University of Melbourne vaccination trials were even less marked than *in vitro*. There were however a couple of exceptions to this: Bowles et al.<sup>86</sup> found that all larvae died at implant sites on 3/10 vaccinated animals; however, this also occurred on 1/10 control animals; Bowles et al.<sup>91</sup> showed an 86% reduction in strikes after 48 h 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).

A number of biological factors will impact on the ability of vaccination to protect against flystrike:

- 1 Stability of antibody at the blowfly wound site and in the blowfly gut:** A blowfly strike wound site would be expected to contain high levels of proteolytic enzymes that would act to degrade host-generated antibodies. These enzymes would be derived from two sources; first, serum exudates produced by damaged host cells, and second, in the excretory/secretory (ES) 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. Sandeman et al.<sup>98</sup> measured the time course of degradation of antibodies at blowfly infection sites on sheep and found that 60% of the antibody in serum exudates at the infection sites was degraded by 6 h after infection, indicating the presence of very active proteolytic enzymes. An examination of the size 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 second aspect of antibody stability is degradation within the gut of blowfly larvae following ingestion. Eisemann et al.<sup>99</sup> showed that ingested antibody remained intact in the anterior portion of the midgut but was degraded significantly in the midsection 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 midsection.
- 2 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<sup>100</sup> described a suppressive effect of larval ES products on sheep lymphocytes. Elkington et al.<sup>101</sup> subsequently described a protein in ES that inhibited lymphocyte proliferation and named it blowfly larval immunosuppressive protein. They demonstrated that blowfly larval immunosuppressive protein binds to the surface of

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 and may act to suppress any immune response generated by vaccination. Hence, Elkington and Mahony<sup>102</sup> suggested that targeting the blowfly immunomodulation mechanism may be a useful component of any strategy for developing a flystrike vaccine.

- 3 *Native versus recombinant antigens:* An issue that impacts anti-parasitic vaccines in general 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 following exposure to native antigens, do not occur, or are greatly reduced, following exposure to recombinant antigens. The native and recombinant antigens can differ in structural properties associated with the folding of the protein and post-translational modifications, most importantly, glycosylation. Yet, recombinant antigens are most likely required if a flystrike vaccine is to be produced in a cost-effective manner, as the recovery of native antigens from larvae is unlikely to be commercially viable. Tellam et al.<sup>94</sup> showed that vaccination with native peritrophin-95 resulted in significant inhibition of larval growth in *in vitro* assays (inhibited 60%) whereas recombinant antigen (produced in both bacterial and insect cell culture systems) resulted in <20% inhibition. Bowles<sup>95</sup> 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 the recombinant antigens had no effect.
- 4 *Antibody-mediated versus cellular response:* 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* study from that time found that protection against flystrike after vaccination was associated with cellular responses rather than antibody-mediated responses.<sup>91</sup> 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. In reviewing these early vaccine trials, Elkington and Mahoney<sup>102</sup> suggested that future efforts in flystrike vaccination should be directed at generating a cellular rather than an antibody-mediated response.

#### Vaccination against fleece rot

Fleece rot is considered to be a major predisposing condition for body strike in sheep. It has therefore been suggested that a means to reduce the incidence of body strike would be to reduce the incidence of fleece rot through vaccination against the bacteria that cause the condition.<sup>103</sup> 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 dermal lesions and the fleece rot-associated odours that are thought to attract flies to the sheep. A programme of work on vaccination against fleece rot was conducted at CSIRO in the 1980s.<sup>103,104</sup> The

work focused on vaccination against the bacterium *Pseudomonas aeruginosa* as a means to control fleece rot and body strike. A series of field flystrike-challenge experiments showed significant levels of protection against body strike after the administration of prototype *P. aeruginosa* vaccines. However, the research programme ended without a vaccine being commercialised.

Vaccination against fleece rot as a means to prevent flystrike faces a number of issues. The studies described above focused on vaccination against *P. aeruginosa*; however, a number of reports have shown that fleece rot is often caused by other bacterial species.<sup>105–107</sup> Kingsford and Raadsma<sup>106</sup> reported the presence of *P. aeruginosa* in only 14% of 646 fleece rot cases in three surveys conducted between 1993 and 1995. In addition, while *P. aeruginosa* can attract *L. cuprina* and stimulate oviposition, a number of other commonly associated bacteria have similar or even stronger effects.<sup>108</sup> A further issue with regard to the width of the spectrum of activity required for a fleece rot vaccine (when considering *P. aeruginosa* alone as the target bacterium) is the fact that up to 14 serotypes have been recognised among the *P. aeruginosa* samples recovered from sheep in Australia.<sup>109,110</sup> Burrell<sup>104</sup> showed that a prototype *P. aeruginosa* vaccine only protected sheep from flystrike if the same serotype of the bacterium was present. In terms of potential effects of fleece rot vaccination on breech strike, Raadsma et al.<sup>111</sup> reported that the incidence of non-body strikes (pizzle, breech and head) was not related to the duration of wetting (artificial or field rain) and that the incidence of non-body strikes was not related to the severity of fleece rot. 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.<sup>112</sup> Therefore, on the basis of current knowledge, the development of a fleece rot vaccine for sheep with a sufficiently wide spectrum of activity to give practically useful reductions in flystrike incidence, seems unlikely.

#### Scouring

Scouring (diarrhoea) 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, and subsequent oviposition at the site leads to the initiation of a strike. The literature on the causes of scouring and association with flystrike was recently reviewed thoroughly.<sup>113,114</sup> Scouring is most important in southern regions of Australia with winter rainfall (south-east Australia) or Mediterranean climates (southern Western Australia). Outbreaks most often occur in winter and early spring when the number of infective nematode larvae on pasture is highest (most importantly, *Trichostrongylus* spp and *Teladorsagia circumcincta*). Nutritional scouring is most commonly associated with young lush pastures and rapidly growing forage that are rich in nonstructural carbohydrates, plant proteins, and macrominerals, particularly potassium. Pasture species implicated as possible causes of scouring include capeweed, forage oats, Phalaris, and various brassica crops. The movement of sheep from pasture to forage crops may lead to scouring due to lag period in the adaptation of rumen microorganisms to a new feed type. Scouring may also be caused by bacterial infections (principally, *Yersinia* and



*Campylobacter*) or protozoa (principally, *Eimeria*, but can also involve *Cryptosporidium* and *Giardia*). Alkaloid toxins produced by endophytes (fungi) associated with perennial ryegrass have also been reported to increase the incidence of scouring in lambs.

Control of scouring due to worms is influenced by an interaction of worm exposure levels and sheep age/immune status.<sup>114</sup> Young sheep show a lower immune response to incoming worms as they have had less time to develop an effective immune response compared to mature sheep, resulting in higher worm burdens and scouring. Lambing ewes experience a transient decrease in immunity to worms, resulting in higher worm burdens and scouring. In mature sheep (>12 months), scouring is not directly related to adult worm burden in most cases. Rather, the scouring (sometimes referred to as 'hypersensitivity scouring') is associated with a heightened inflammatory response to incoming third- and fourth-stage larvae in mature sheep that have acquired a higher degree of worm immunity than young sheep through previous exposure to worm larvae. This type of scouring 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 and hence is related to the degree of previous exposure to worms

The use of anthelmintics to control worms, and grazing management strategies to reduce exposure to worms, can reduce scouring that is due to high worm burdens. Anthelmintic treatment in response to elevated worm egg counts, or observed scouring in lambs, requires the use of an effective drench product to which the worms on the property are not resistant. The WormBoss site (<http://www.wormboss.com.au/sheep-goats/>) describes resistance-testing services for identifying anthelmintics likely to be effective: DrenchCheck (single product) or DrenchTest (faecal egg count reduction test; multiple products). The WormBoss website also provides advice for graziers in different regions of Australia on the preparation of low worm-risk paddocks in order to reduce the intake of larvae from pasture.

Studies have indicated that dagginess (dag scores) in mature animals is heritable and hence genetic selection for reduced scouring should be possible (described in more detail below), and culling of ewes with repeated dag is recommended (WormBoss). An Australian Sheep Breeding Value (ASBV) for dag is available through Sheep Genetics (<https://www.sheepgenetics.org.au/>). However, there are a number of issues that impact on the uptake by industry and usefulness of this measure, including, inconsistency of scouring year-to-year and in different environments, the need to allow sheep to scour without management intervention in order to achieve expression of variation in the population, and the need to assess dag scores on animals at a later age than for the other flystrike-related traits such as wrinkle and breech cover. Importantly, it should be noted that separate ASBVs are available on the Sheep Genetics database for worm resistance and scouring and that programmes focusing on selection for increased resistance to intestinal nematodes (low faecal egg counts) will not reduce the prevalence of scouring.<sup>115</sup> 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.<sup>114</sup>

Prevention of low worm burden scouring in mature animals would also be aided by an increased understanding of the mechanisms responsible for the phenomenon. The need for investigation of a number of factors associated with this type of scouring was highlighted by Jacobson et al.,<sup>113,114</sup> 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 selection 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, that is, the extent and timing of previous worm exposure required before the scouring response is triggered.
- 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).

### Breeding

Breeding more resistant sheep was one of the approaches considered to countering the blowfly problem in the early stages of the sheep industry, and the current general consensus is that this will be key among approaches for controlling flystrike in the future. 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.<sup>116–119</sup> However, because the incidence of flystrike is often low or intermittent and management is geared to suppress the occurrence of the strike, direct selection against strike is often inefficient. Hence, the identification of indirect characters associated with flystrike is critical to the development of effective selection programs.<sup>120</sup> There have been many reports describing the identification of indirect characters for breech strike and body strike resistance, and as these have been thoroughly reviewed elsewhere,<sup>5,117,119,121–125</sup> we focus here on only the key characters.

#### Breech strike

The role played by breech and tail folds in susceptibility to breech strike was recognised very early in the emergence of flystrike as an important problem in Australia.<sup>39,126</sup> Seddon and Belschner<sup>127</sup> provided a detailed description of features of the various skin folding patterns in the breech and around the tails of sheep and discussed their role in determining susceptibility to strike. However, with the development of the Mules operation in the 1930s and its ability to provide a high level of protection against the breech strike, momentum in breeding breech strike resistant animals was lost and research became more focussed on improving techniques of mulesing and tail treatments.<sup>128</sup> It was not until the establishment of the Australian Wool Innovation breech strike resource flocks in the winter rainfall (Mediterranean) climate of Western Australia (WA) and in the summer rainfall environment at Armidale in NSW that significant further research into breeding for resistance to breech strike resumed.<sup>118,119,129–132</sup> These studies have confirmed the overwhelming

importance of breech wrinkle in susceptibility to breech strike in present-day Merino types, both in the Mediterranean climate in WA and in the summer rainfall zone of NSW, and have focussed on identifying other factors associated with resistance and optimal methods for incorporating selection for breech strike resistance into breeding programs.

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.<sup>133,134</sup> Various attempts have been made to develop breech modification techniques that both remove wrinkles and provide such an area of bare skin while being more humane than mulesing, for example, plastic breech clips, or topical and intradermal administration of chemicals and bioactive agents to cause skin stretching and depilation.<sup>135</sup> These have generally proved to be unacceptable on welfare grounds or not sufficiently effective to provide a practical alternative to mulesing.<sup>135–140</sup> However, given the known importance of bare skin at the breech, there has also been interest in genetically increasing the area of bare skin utilising across-breed variation<sup>141,142</sup> and by selecting within populations for extreme phenotypes such as that reported in a number of merino flocks.<sup>143</sup> Edwards et al.<sup>143</sup> showed that the heritability of this bareness trait was moderate to high and there were no significant unfavourable associations with other economically important traits identified. Subsequent studies have confirmed that crutch and breech wool coverage, assessed using Visual Sheep Scores (<https://www.wool.com/globalassets/wool/sheep/welfare/breech-flystrike/breeding-for-breech-strike-resistance/visual-sheep-scores-producer-version-2019.pdf>), are both heritable and genetically associated with breech strike resistance.<sup>130,131</sup>

The role played by urine and faecal staining of the breech wool in susceptibility to breech strike has changed somewhat from the conditions prevailing when the early breech strike research was conducted. Beginning in the 1940s, there was an 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 the breech strike.<sup>2</sup> In addition, there was an increasing use of radical mulesing, often with a shorter tail, during this period.<sup>128</sup> The importance of the different management systems on breech strike predisposition was indicated by Watts et al.<sup>2</sup> They noted that in radically mulesed Merino ewes, scouring was the main predisposing factor for breech strike, whereas in unmulesed ewes, the breech strike was invariably associated with urine staining. 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. In the southern flocks, wrinkle and dag score were consistently the two main characters associated with the occurrence of breech strike.<sup>131</sup> In the WA resource flock, the genetic correlations between dag score and breech strike were high, and dag score was moderately to highly heritable.<sup>130</sup>

One of the largest barriers to selection for breech strike resistance is the undesirable correlation with production traits, in particular, fleece weight and fibre diameter. Richards and Atkins<sup>144</sup> confirmed that fleece weight will be reduced if single-trait selection for wrinkle is applied. However, they also noted that if the wrinkle is included in a carefully designed selection index, then breech wrinkle can be

reduced with little associated reduction in rates of gain in production traits. They also indicated that the accuracy of selection and rates of gain can be increased by using the correlated and more readily-assessed characters, neck wrinkle and body wrinkle together with breech wrinkle, and by using breeding values available from SHEEPGENETICS, which also utilise information on the performance of relatives. Hatcher and Preston<sup>145</sup> examined the phenotypic associations of breech wrinkle and breech cover with key production traits and, although they found a negative association between wrinkle traits and wool production, they also found a favourable correlation with live weight and a number of wool quality traits. The breech cover had a similarly favourable correlation with live weight, but no significant phenotypic associations with other wool production or quality traits. Brown et al.,<sup>146</sup> using data from SHEEPGENETICS, similarly indicated some significant antagonisms between wrinkle score and production traits. However, they also showed that using index selection, reductions in breech wrinkle score could be achieved over a 10-year period while maintaining reasonable rates of genetic gain in production traits.

Brien et al.<sup>147</sup> extended these studies by including all three of the main indirect criteria for which ASBVs are available in MERINOSELECT (breech wrinkle, dag and breech cover) and examined the rates of gain in flystrike resistance that could be made by adding flystrike as a trait to three MERINOSELECT indices: 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. They concluded 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. Importantly though, Walkom and Brown<sup>148</sup> noted that one of the key factors holding back the incorporation of resilience and resistance traits, such as flystrike resistance, into formal breeding indices is the derivation of accurate economic values. An early start towards this end was made for fleece rot and bodystrike,<sup>149</sup> but further work in this area is needed for body strike and more particularly, for breech strike resistance.

### Body strike

The association between fleece rot and body strike was recognised as early as 1931.<sup>126</sup> Subsequently, Hayman<sup>150</sup> demonstrated that resistance to fleece rot was a heritable trait. Atkins and McGuiirk<sup>120</sup> 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 body strike are somewhat intermittent and the incidence of fleece rot is generally higher than body strike, and because management is geared to suppress the expression of body strike, selecting on the basis of fleece rot was considered likely to provide faster gains in body strike resistance than direct selection.<sup>120,151</sup> 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.<sup>117,124</sup>

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), and immune response (both to challenge by blowfly larvae and to fleece rot bacteria). All of these characters have been reviewed elsewhere and most have shown association in some flocks or conditions but not in others.<sup>121,122,124</sup> The two characters most consistently related to body strike, although not in all flocks, appear to be unscoured wool colour and fibre diameter variability.<sup>152–155</sup> An ASBV for fleece rot is now available in MERINOSELECT and can be utilised in breeding programs to assist selection for resistance to body strike.

### Genomic breeding values

Genomic selection, whereby the presence of major genes, groups of genes or genomic indices are utilised to predict the 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 the production environment or seasonal conditions (e.g. high or low flystrike environments, and high or low flystrike risk year).

Currently, research in this area is in its infancy for flystrike-related traits. Raadsma et al.<sup>156</sup> and Engwerda et al.<sup>157</sup> 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 flystrike-related differences. Pickering et al.<sup>158</sup> reported a number of immune, diarrhoea and wool growth genes were associated with flystrike and dag score, and Bolormaa et al.<sup>159</sup> 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 the breech strike or body strike susceptibility was reported. More recently, an attempt at finding genomic associations for variation in breech strike resistance, utilising data from the WA and NSW breech strike selection lines, found only single nucleotide polymorphisms (SNPs) of small effect.<sup>160</sup> However, the report indicated that even though no SNPs of large effect were found, the aggregation of the small effects of many SNPs might be effective in the creation of genomic enhanced breeding values.

The development of a training population for the estimation of genomic breeding values for flystrike resistance requires the development of a large population of sheep that are phenotyped for flystrike and genotyped. Establishing a purpose-designed flock to accomplish this is expensive. An approach used in other areas has been to integrate data collected from existing genetic evaluation programmes and other experimental 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>). Greeff et al.<sup>161</sup> also 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 the breech strike. It should also be possible to use judicious contributions from commercial or other research flocks to add to this database, as already sought for other traits in the MLA resource flock, without having to institute deliberate flystrike challenge testing. Drawing information from a wide range of sources in this way currently seems the most pragmatic way of assembling the large database required to establish reliable genomic values.

In conclusion, it is widely recognised in the sheep industry that breeding for reduced susceptibility to flystrike is the most appropriate and effective long-term solution to the issue of flystrike. We suggest that there are a number of initiatives that should be implemented, or emphasised to a greater degree than is presently the case, in order to hasten the rate at which breeding can influence flystrike susceptibility more widely across the sheep industry:

- collection of more industry data on the major flystrike traits (dag, breech cover and breech wrinkle) in order to increase the accuracy of ASBVs for these traits and their applicability to different industry breeding objectives and management regimes. Means to encourage more widespread phenotyping of breech characters and submission to Sheep Genetics should be explored. 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. Development of a urine stain ASBV could also be considered, with a directive in the Visual Sheep Scores booklet to score urine stain at times of low dag/scouring to help increase the accuracy of assessment of this trait. The most appropriate use of urine stain as either a selection tool or an independent culling tool should also be considered.
- there is a need to facilitate practical ‘useability’ of breech strike traits in MERINOSELECT. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. 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 in order to reduce breech strike susceptibility, while maximising gains in other traits, should be distilled into a fact sheet available from the Sheep Genetics website.
- there is a need to establish a ‘virtual’ genomic resource flock, based around that MLA Genomic Resource flocks, which can incorporate data drawn from a wide range of research and commercial flocks, towards the development of genomic enhanced breeding values, as has previously been suggested.<sup>162</sup>

### Trapping

As early as the 1930s, Mackerras et al.<sup>163</sup> showed that high-intensity trapping with blowfly traps baited with offal and sodium sulphate

could bring about a reduction in flystrike incidence. Since this time, there have been many studies to design new and better traps and modified attractants to improve the effectiveness and practical utility. Anderson et al.<sup>164</sup> developed a larger scale trapping system based on portable 'wheely bins' baited with a sheep carcass or offal treated with sodium sulphide. 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 the selectivity of the traps by allowing entry by *L. cuprina* but blocking access of larger blowflies. The bins achieved a degree of adherents and were relatively commonly used by growers, particularly in the more extensive sheep production areas of Australia. The usefulness of offal-baited traps was also assessed in New Zealand, with mixed results.<sup>165–167</sup> 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* in the field by removing competition from other species breeding in carcasses. A number of alternate baits have been developed or tested to enhance the efficacy, specificity and utility of trapping: a mixture of *Proteus mirabilis* and gut mucus, or liver sodium sulphide,<sup>168,169</sup> freeze-dried liver,<sup>170</sup> a synthetic bait consisting of sulphur containing volatile compounds encapsulated in a slow-release casein matrix,<sup>171</sup> cloth targets impregnated with sucrose and 10% triflumuron, a growth regulator insecticide.<sup>172</sup>

Most notable among 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.<sup>173,174</sup> Once uncapped, wicks in the traps emit the attractants into the air for up to 6 months. Recommendations were to use these traps at a rate of 1 per 100 sheep. Trials consistently demonstrated a reduction in *L. cuprina* populations when the LuciTraps were used according to instructions.<sup>173–176</sup> 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 flocks.<sup>173,176</sup> A comprehensive study conducted in 2003, comprising four separate experiments over 3 years, indicated a reduction in flystrike incidence of between 38% and 55%.<sup>177</sup> However, Lucitraps 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 to keep the traps functionally effective. 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 in the reduction of strike incidence.

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.<sup>178</sup> The notion of a threshold below which the incidence of the strike is determined primarily by fly numbers, and above which the number of susceptible sheep is the major strike limiting factor,<sup>163,179,180</sup> appears to support this proposition. In addition, McKenzie and Anderson<sup>178</sup> demonstrated that early-season insecticide treatment of sheep prior to *L. cuprina* emergence from overwintering, which 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 after flystrike risk becomes apparent. 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, it has not been experimentally validated. However, where trapping is to be used, it seems critical that it 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.

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 overwintering blowfly population first emerges, and can assist in timing sheep treatments, or perhaps the implementation of strategies such as early-season insecticide treatments. In addition, a rapid increase in blowfly numbers can be indicative of the commencement of strike waves. 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.<sup>181</sup> It is likely 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.

#### Forecasting and detection of strikes

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 regularly treat prophylactically 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.<sup>181</sup> described 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, rainfall, temperature and wind speed, and fleece remaining moist for at least 24 h to enable egg hatch and larval survival. 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 the use of this system for detecting body strike outbreaks, it was not widely adopted, particularly in areas where breech strikes were frequently the main problem.

With a view to the development of better prediction of flystrike to help producers optimise their control options, and towards a better understanding of the factors that regulate the incidence of flystrike, Wardhaugh and Morton<sup>180</sup> modelled the incidence of flystrike in the Shoalhaven valley in NSW. They demonstrated that the weekly incidence of flystrike was related to the abundance and activity of gravid flies and various measures of temperature, rainfall and pasture growth. However, the model they developed took no account of the age or sex of the sheep struck or of the variable effects of flock management on sheep susceptibility. Follow-up studies using large-scale flock monitoring programs aimed to clarify the roles of animal

husbandry, weather and fly abundance in determining strike incidence in different regions and classes of sheep.<sup>182</sup> This study found that the base model required only daily rainfall, mean daily temperature, and relative humidity at 9.00 am for prediction and did not require knowledge of fly density or fly activity to provide an acceptable standard of prediction. An alternative approach to predicting flystrike was taken by Ward<sup>183</sup> 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 was positive. He suggested that a useful early warning system could be developed based on the significant correlation between flystrike incidence and the southern oscillation index up to 6 months earlier. Whether this association is also apparent in data from areas outside of Queensland, or whether the correlation calculated could provide practically useful accuracy of strike prediction, does not appear to have been assessed.

Wardhaugh et al.<sup>182</sup> noted that the models based on historical climate data could have significant value for strategic planning and for developing decision support systems for growers, for example aiding growers to optimise the time 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, the timing of chemical treatments and the effect of breeding in reducing susceptibility to strike (<http://www.flyboss.com.au/sheep-goats/tools/flystrike-quick-tools-online.php>).<sup>184</sup> One significant improvement made in the FlyBoss models is the more accurate localisation of predictions based on the use of the Silo climate database (<https://www.longpaddock.qld.gov.au/silo/>). The FlyBoss models can assist producers to optimise their current flystrike control programs, or perhaps to modify their programs to reduce reliance on mulesing, or to take account of insecticide resistance in fly populations 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,<sup>185</sup> 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,<sup>186</sup> and to assess the financial and management implications of using a strategic early treatment approach to the application of preventative chemicals.<sup>187</sup> It should be kept in mind, however, that the timing of strike waves is largely determined by weather conditions and, hence, to a large extent, the ability to predict strike waves accurately and early enough to implement emergency preventative controls remains heavily dependent on the accuracy of weather forecasting.

Flystrike management can be divided into 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' to monitor mobs for flystruck sheep can account for a significant part of flystrike management costs when fully budgeted. If strikes are not detected early, sheep develop fever and cease feeding, and death may result within 6 days.<sup>188,189</sup> Early detection of flystrike is difficult and

continual monitoring of flocks is required to enable timely treatment of struck sheep. Accurate methods of predicting strike outbreaks would allow more strategic application of preventative flock treatments if the 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, kicking and trying to bite the affected area.<sup>190</sup> 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. In some flocks, there is a high incidence of covert strikes that are only detected by intensive inspection of the sheep<sup>191</sup> and therefore present a problem for early detection of strikes based on visual inspection. Despite the labour costs involved in monitoring flocks for clinical signs of the strike, there has been little investigation of alternative approaches to manual inspection. Cramp et al.<sup>192</sup> examined the potential of using electronic nose (E-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 and also detect flystrike odour on the day of larval implantation in three of four experiments. Furthermore, periods of 'sniffing' as short as 2 s and sensors placed 0.7 m away from the sheep both gave accurate discrimination of strike. The authors noted that with the rapid advances currently being made in E-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,<sup>193</sup> warranted further investigation. Grant et al.<sup>194</sup> examined video footage of struck and unstruck sheep and confirmed that both qualitative and quantitative assessments identified behavioural differences between them. They suggested that remotely assessed behaviour could provide a low-input method for identifying animals that require treatment. The authors also indicated the advances that have been made in the development of biosensors to detect behavioural changes in a number of livestock species including pigs and dairy cattle<sup>195, 196</sup> and suggested that similar possibilities exist for the detection of flystrike in sheep.

#### Genetic manipulation of the fly population

Autocidal control aims to bring about area-wide suppression or eradication of a pest population by the release of insects of the same species that have been modified to confer sterility or cause genetic death. The SIT 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. Utilisation of this approach requires the establishment of significant infrastructure ('factories') to rear the large numbers of flies required, development of a release strategy (usually from aircraft), and desirably, technology to separate or incapacitate the female flies from the release population. In its most well-known use, the SIT method was successfully used to eradicate New World Screwworm flies from North and Central America.<sup>197</sup> This method has also been used to eradicate regional incursions of insects, such as fruit flies in fruit fly-free areas of Australia, an incursion of screwworm flies in Libya, and tsetse flies from the Island of Unguja in the Zanzibar archipelago.<sup>198</sup> 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.

As an alternative to SIT, in the 1970s CSIRO investigated the use of a 'field female killing' (FFK) strain of the blowfly to suppress or eliminate blowfly populations.<sup>199</sup> A key difference to SIT was that the genetic control mechanism would be passed onto successive generations to allow for spread through a population rather than just relying on the serial release of sterile flies. Females of the CSIRO strain were homozygous for an eye colour mutation resulting in white eyes and functional blindness that are lethal to the females once they are released into the field. The male flies are not blind; however, they are semisterile and mate with wild-type females in the field to pass on the mutation. Fly populations are suppressed in two ways: first, only a proportion (approximately 50%) of the eggs hatch, and second, 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<sup>2</sup>), off South Australia, in 1985–1986 was successful in suppressing the blowfly population to undetectable levels; however, 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 that immigrated or were inadvertently reintroduced from the mainland. A subsequent trial conducted on the Furneaux Islands in Bass Strait (main landmass Flinders Island, area = 1367 km<sup>2</sup>) 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 field flies.

Advances in molecular biology techniques, including the development of gene-editing technologies, and the recent availability of the sheep blowfly genome<sup>17</sup> provide the potential for more elegant systems of genetic control, such as release of insects with dominant lethality (RIDL),<sup>200</sup> or potentially using gene drives to spread deleterious genes through fly populations. Notably, the RIDL system, which has been developed for control in mosquitoes,<sup>201</sup> is very similar in principle to the FFK strain developed earlier by CSIRO, but whereas the CSIRO strain used recessive mutations to confer lethality, the RIDL males carry a dominant female-lethal gene. In addition, the development of repressible female lethal systems that allow for the normal reproduction of female flies within the mass rearing

facilities, but that are lethal when the flies are released, can significantly reduce the costs of production of flies for release and reduce the likelihood of inadvertent release of fertile female flies.<sup>202</sup> Such repressible systems, based on the presence or absence of tetracycline from the larval diet, have been developed for *L. cuprina*.<sup>203–205</sup> More recently, embryonic-specific elements have been added into the female-lethal tetracycline system.<sup>206, 207</sup> Absence of tetracycline in the fly diet in the insect rearing facility results in the death of females at the very early embryonic stage, thus avoiding the production and release costs for the unwanted females. 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. These advances in molecular manipulations of blowflies have provided opportunities to generate flies that may be suitable for genetic control programmes based on the release of fertile male flies carrying female lethal genetic systems.

Gene drives are an immensely powerful tool that can allow targeted genes to preferentially spread through a population.<sup>208</sup> Gene drives have been identified in nature, for example, homing endonuclease genes (HEGs) in bacteria,<sup>209</sup> and these bacterial genes have been introduced into mosquitoes. More recently, the advent of CAS/CRISPR and associated technologies has enabled the design of purpose-designed gene drives to target critical genes in pest populations, providing a range of new possibilities.<sup>59</sup> Homing endonuclease genes 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.<sup>210</sup> Clearly, the use of gene drives would enable the design of genetic control strategies that could help overcome the logistic and cost 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. However, gene drives, once introduced to a population can spread by themselves and there are serious concerns about their unpredictability.<sup>211,212</sup> 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 have unpredictable consequences. 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.<sup>212</sup> 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.<sup>212,213</sup> A full consideration of gene drives is beyond the scope of this review, however, further information on the issues surrounding the potential use of gene drives is provided by Deardon et al.<sup>211</sup>

The success of the sterile male approach in eradicating New World Screwworm fly from the north and central America was partially due to the particular geography of this area and the fact that the fly died out through most of North America during winter. There was only a small area in the eastern United States, in southern Florida where

flies survived the winter, and this was initially targeted for eradication using strategic chemical applications together with the sterile male release. In the western areas of the United States, 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 present a significant hurdle for an area-wide genetic approach. A possible exception is in Tasmania, or other geographically bounded areas such as Western Australia, or other islands such as Kangaroo Island in South Australia.<sup>214</sup>

An extensive economic evaluation of the economic feasibility of CSIRO's FFK technique for the genetic control of the sheep blowfly in Australia was carried out by King et al.<sup>214</sup> 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 areas, 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 a barrier zone between the pastoral zone and the more easterly sheep production zones. Overall, it was concluded that the return on investment looked favourable for larger, higher sheep density areas if problems associated with the large-scale rearing of the FFK strain, evident at that time, could be overcome. However, current costs of production and sheep and wool prices are very different from those at the time of this analysis, while recent technological developments that affect costs of rearing and distributing flies could have a major effect. Current sheep numbers in Australia are very much lower than the 170 million sheep population at the time of the earlier study, and the proportion of Merinos, which are a greater strike risk than meat-producing breeds, is also much lower than in 1991. Clearly, a reassessment of the economic feasibility is needed before embarking on any autocidal approach. In addition, implicit in the assessment made by King et al.<sup>214</sup> was that such an approach would be funded with a grower levy. The political will and likely grower response to the imposition of such a levy would be a key consideration and precedents suggest that funding with a voluntary levy is unlikely to be tenable.

#### Recommendations on future research and adoption pathways

The present review has highlighted a number of areas that we suggest warrant consideration in order to provide long-term solutions to the issue of flystrike. We have described these in some detail in the various sections of the review and list them here. 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.

Areas for further research:

- insecticide resistance management: insecticide resistance diagnostics, modelling of insecticide-use strategies.
- novel delivery methods for chemical and biological agents: emphasis on CR technologies and nanotechnology to provide extended periods of protection; the potential for systemic delivery of prophylactics.
- development of more readily measurable breeding indices for flystrike-related traits, including genomic breeding values.
- the need for a greater understanding of the basis for low worm burden scouring in mature animals.
- increased understanding of the feasibility of area-wide eradication or suppression of sheep blowfly populations utilising new genetic manipulation technologies such as CAS/CRISPR, or through the use of *Wolbachia*.
- improved flystrike prediction models to extend decision support tools for wool producers.

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

- insecticide resistance management strategies: optimal insecticide use, including drug rotations and timing of husbandry interventions (crutching and shearing) with insecticide use; greater emphasis on the use of existing resistance diagnostics.
- guidelines for breeders on how to best use current flystrike-related ASBVs.
- management practices (including breeding and optimal anthelmintic use) to reduce scouring.

The cloning of the blowfly genome may be useful in providing impetus to some flystrike control strategies, such as the development of resistance diagnostics, identifying new insecticide and vaccine targets and designing area-wide approaches that seek to directly suppress or eradicate sheep blowfly populations. However, in this review, we have highlighted commercial, biological, feasibility and societal factors that may act to temper the potential for the genome to act as the basis for providing some control options.

Overall, we envisage a future in which long-term control of flystrike is provided by breeding sheep that are less susceptible to strike, with increasing use of genomic breeding technologies to facilitate selection, and with a greater understanding of means to reduce scouring. The increasing globalisation of the veterinary pharmaceutical industry and the relatively small size of the sheep ectoparasite market on the world stage is likely to slow the future flow of new chemistries onto the market. This will increase the requirement for the judicious use of chemicals, with emphasis on optimal delivery and use patterns to increase periods of protection and minimise the development of insecticide resistance. These strategies would be supported by a greater ability to utilise online flystrike prediction and decision-support tools to manage all aspects of sheep production relevant to flystrike control.

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