

# Dicyclanil resistance in the Australian sheep blowfly, *Lucilia cuprina*, substantially reduces flystrike protection by dicyclanil and cyromazine based products



Narelle Sales\*, Monica Suann, Kim Koeford

NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Private Bag 4008, Menangle, Narellan, 2567, NSW, Australia

## ARTICLE INFO

### Keywords:

Flystrike  
Dicyclanil  
Resistance  
Reduced protection  
Cyromazine

## ABSTRACT

Late in 2017, field samples of the Australian sheep blowfly, *Lucilia cuprina*, were submitted by sheep producers from three states of Australia (South Australia, Victoria and New South Wales). Some were collected by submitters concerned about shortened periods of flystrike protection from dicyclanil based products. Neonate larval offspring from the NSW field samples survived and successfully completed their life cycles following exposure to dicyclanil and cyromazine at susceptible discriminating concentrations *in vitro*. The *in vivo* study reported here used dicyclanil resistant neonate larvae to assess the flystrike protection provided by a cyromazine jetting fluid and a number of dicyclanil based spray-on products, when applied to sheep six weeks after shearing.

The two dicyclanil resistant blowfly strains used in this study showed *in vitro* resistance ratios, at the LC50, of approximately 13- and 25-fold relative to a dicyclanil and cyromazine susceptible strain. Compared to the levels of resistance that *L. cuprina* has developed to other insecticides these are relatively low, however, three dicyclanil based spray-on products (active ingredient 12.5 g/L, 50 g/L and 65 g/L) had protection periods reduced by 73%, 78% and 69% respectively when compared to the maximum protection periods claimed by the manufacturer. A 50% and a 33% reduction in protection period was also observed to a cyromazine and an ivermectin based jetting fluid respectively. In contrast, protection periods were attained or exceeded regardless of the treatment used against field derived dicyclanil susceptible neonate larvae.

For the first time we confirm that dicyclanil resistance enables the completion of the *L. cuprina* life cycle following flystrike initiation on dicyclanil or cyromazine treated sheep when insecticide levels are considered high and protective. This study also provides *in vivo* information on the effect of dicyclanil resistance on the protection provided by a product with an active ingredient belonging to an unrelated insecticide group. Dicyclanil resistance is of major concern to the Australian sheep industry.

## 1. Introduction

Flystrike, or cutaneous myiasis, is an economically important disease of sheep. It has been estimated to cost the Australian sheep industry \$173 million dollars per annum when treatment, prevention and production losses were considered (Lane et al., 2015). To prevent flystrike, Australian sheep producers have relied largely upon the long periods of protection provided by the insecticides dicyclanil and cyromazine. These insecticides have a similar chemical structure, have been used for 21 years (Bowen et al., 1999) and 41 years (Hart et al., 1979) respectively and are seen as critical to current on-farm management practices by many sheep producers. Cross-resistance between cyromazine and dicyclanil was reported in the sheep blowfly and *Drosophila melanogaster* following mutagenesis and *in vitro* assessment (Magoc

et al., 2005). It was also confirmed *in vitro* in a naturally occurring Australian population of *Lucilia cuprina* in 2011 (Levot, 2012). A subsequent survey established that 62% of submitted blowfly populations (n = 58) had what was described as “low level” resistance to cyromazine (Levot, 2014). Of those resistant to cyromazine, only 22% (n = 8) also displayed “low level” cross-resistance to dicyclanil. Low level resistance was defined *in vitro* as a strain which survived the cyromazine susceptible discriminating concentration (SDC) but not eight-fold the SDC. Survival of eight-fold the cyromazine SDC was considered by Levot et al. (2014) as the worst-case scenario following intense selection pressure unless a second resistance mechanism evolved to dicyclanil. At that time, an *in vivo* study by Levot et al. (2014), using a strain with eight-fold resistance to cyromazine and three-fold to dicyclanil, established that the periods of protection provided by both cyromazine

\* Corresponding author. NSW DPI, Elizabeth Macarthur Agricultural Institute, Private Bag 4008, Narellan, NSW, 2567, Australia.

E-mail addresses: [narelle.sales@dpi.nsw.gov.au](mailto:narelle.sales@dpi.nsw.gov.au) (N. Sales), [monica.suann@dpi.nsw.gov.au](mailto:monica.suann@dpi.nsw.gov.au) (M. Suann), [kim.koeford@dpi.nsw.gov.au](mailto:kim.koeford@dpi.nsw.gov.au) (K. Koeford).

<https://doi.org/10.1016/j.ijpddr.2020.04.005>

Received 18 February 2020; Received in revised form 27 April 2020; Accepted 29 April 2020

Available online 11 May 2020

2211-3207/ Crown Copyright © 2020 Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and dicyclanil based products were reduced. In this study, larvae were classified as alive or dead at 24, 48- and 72-h post implant. In comparison, Baker et al. (2014) stated the protection periods were unaffected by the previously described cyromazine resistance (Levot, 2012) if applied according to the manufacturer's instructions. The cyromazine resistant strain used in this *in vivo* study was assessed as having 4% cyromazine resistant individuals and was not pressured during the study period. To assess the protection period, Baker et al. (2014) used a modified implant technique and adopted a scoring system for long-term larval viability based on subjective assessment at 24, 48- and 72-h post implant.

Any reduction in the protection period provided by flystrike prophylactic treatments is of major economic and animal welfare concern to the Australian sheep industry. Therefore, interested producers collected larvae from struck sheep and submitted them to our laboratory for testing. We found concurrent resistance to cyromazine and dicyclanil in all populations ( $n = 10$ ) submitted by NSW sheep producers from late spring 2017 to autumn 2018. The aim of the present study was to determine *in vivo* the current levels of protection provided by both dicyclanil and cyromazine based products on sheep when challenged with these recently collected dicyclanil resistant strains from NSW.

## 2. Material and methods

### 2.1. *Lucilia cuprina* strains

*L. cuprina* larvae that were collected from sheep by producers across three states in Australia, during the late spring (2017) through to autumn (2018) fly season, were established in culture. The submissions received from South Australia and Victoria were from properties which indicated that only cyromazine based products were routinely used. Conversely, the NSW submitters specified the current exclusive use of dicyclanil based products but did state they had used cyromazine prior to the release of dicyclanil to prevent flystrike.

A proportion of the first instar larvae obtained from the 2nd generation of each strain were exposed *in-vitro* to technical grade dicyclanil and cyromazine at susceptible discriminating concentrations (SDC) (Yen et al., 1996) to determine their resistance status. A pooled dicyclanil susceptible strain (DSus) was formed from those submissions which failed to survive the SDCs of both dicyclanil and cyromazine. It consisted of four strains, submitted from SA and Victoria, and was cultured without insecticides. The two dicyclanil resistant strains (DRes) consisted of nine strains submitted from NSW. Three strains had a mean survival of 48% at the SDC and survivors at four times the dicyclanil SDC (DRes4). The remaining six strains had survivors at eight times the dicyclanil SDC (DRes8) and a mean survival of 62.3% at the SDC, of which one strain had 90.2% survival. Once formed, these two DRes composite field strains were cultured on food sources containing 4 times and 8 times the dicyclanil SDC, respectively. DRes4 and DRes8 were selected in this way for three generations prior to commencement and throughout the implant challenge, a total of ten generations. However, the strains were not selected for one generation prior to each implant occasion. The concentration of dicyclanil used to select these two strains remained constant over time, aiming to eliminate dicyclanil susceptible types rather than applying increasing selection pressure to increase resistance levels (Hughes and Shanahan, 1978., Levot, et al., 2014). The neonate larvae from DRes4 and DRes8 were combined in a 50:50 ratio for implantation.

### 2.2. Animals

In August 2018, a flock of Merino yearlings, which had been crutched and drenched in January 2018, was inspected and 72 wethers selected for this trial. The selected sheep were weighed (mean body weight  $39.25 \pm 1.7$  kg), their ear tag numbers recorded and were

returned to pasture as a discreet research flock. These sheep had never been treated with an insecticide and were assumed to be immunologically naïve to flystrike based on their animal husbandry and rainfall records which indicated that only 41.4% of the mean annual rainfall had been received in the previous 12 months (Australian Government: Bureau of Meteorology; <http://www.bom.gov.au/climate>).

The 72 ear tag numbers were placed in order according to weight and randomly assigned to one of six groups using an on-line random number generator (Random Integer Generator; <https://www.random.org/integers/>). The sheep then received an additional coloured tag which identified them to their designated treatment group.

Following treatment, six weeks after shearing, the sheep were maintained in their treatment groups and housed in outside runs. These runs were small rectangular paddocks which each contained a three-sided skillion shed, feed troughs and water troughs. Each run was separated by a three-vehicle wide laneway at the front and back and a 1 m wide laneway at each side to exclude contact between treatment groups. The sheep were moved to a shed as discreet groups to avoid rain. They were fed daily on a custom sheep pellet ration with added lucerne throughout the study (Vella Stock Feeds, Plumpton, NSW).

### 2.3. Treatments

All treatments were applied six weeks after shearing. The groups were shorn and treated in a staggered fashion to accommodate each products' protection period and to allow the groups to be aligned for simultaneous challenge by both dicyclanil susceptible and resistant larvae.

Prior to treatment the sheep were reweighed and treated on the backline and breech according to the manufacturers' instructions for their individual body weight. The treatment groups were: (a) 12.5 g/L dicyclanil, (CLiKZiN Spray-On™), nine sheep with an average weight of 45.1 kg; (b) 50 g/L dicyclanil (CLiK™ Spray-On), fifteen sheep with an average weight of 48.4 kg; (c) 65 g/L dicyclanil (CLiKExtra™ Spray-On) fifteen sheep with an average weight 44.2 kg; (d) 500 g/L cyromazine (Vetrazin™ Liquid) applied by hand jetting to nine sheep with an average weight of 47.3 kg (Elanco Australasia Pty Ltd, Ryde, NSW); (e) 16.0 g/L ivermectin (Coopers® Blowfly and Lice Jetting Fluid) applied by hand jetting to nine sheep with an average weight of 46.8 kg (Coopers Animal Health, Intervet Australia Pty. Ltd, Macquarie Park, NSW); and (f) untreated controls, fifteen sheep with an average weight of 45.1 kg. The spray-on treatments were applied using the supplied, handheld gun, strictly according to the manufacturer's instructions for dose and spray pattern. Jetting fluids were mixed in a 200L sump according to the manufacturers' instructions and applied using a Dutjet jetting wand (NJ Phillips Pty Ltd, Somersby, NSW) supplied by an electric pump which delivered 650 kPa. Applications were timed, ensuring each animal had an average of 3.3 L of jetting fluid delivered to body and breech. The sheep were then placed into a designated pen inside a shed for 24–48 h until thoroughly dry before returning to their runs. The possibility of insecticide transfer between groups was eliminated as groups did not come in direct contact and only specifically allocated pens and runs were used. The groups were also handled in a defined order, starting with the untreated controls and ending with the group treated with the highest concentration of dicyclanil (65 g/L). The sheep were moved back to their designated pens in the shed to avoid rainfall and during the implant process.

### 2.4. Larval implant technique

To ensure neonate larvae were available for implanting the following morning, cages of blowflies were egged over a 2-h period in the evening and the eggs were kept at approximately 21 °C overnight. The technique used, known as a larval implant, was modified from those previously described (McLeod, 1937, Hughes and Shanahan, 1978,

Holdsworth et al., 2006). Briefly, after parting the wool at an implant site, the skin was lightly scarified and the area moistened with water. A moist cotton wool plug, with approximately 200 neonate larvae on its surface, was placed above the prepared area. The wool was closed over the implant and held in place by a bulldog clip. Five implant locations had been identified along the length of the body from the withers to the rump, one of which was selected randomly prior to each implant occasion. Six sheep from each treatment group received an implant directly under the treatment area from the DSus strain and another from the DRes strain on the opposite side of the animals' midline at the same implant location. An implant roster governed which six sheep from each group would be implanted and as the trial progressed the available implant locations became limited. As a result, the random selection of the implant location ceased and the location which was available on all six sheep of the group was utilized at the latter stages of the study. The sheep implant roster ensured each animal was only implanted on a maximum of four occasions during the study period.

In this study, each implant was assessed at 24, 48 and 72 h except for those on the untreated control sheep. This group had live larvae collected from them after 24 h, the site was clipped, and tincture of iodine applied in line with animal welfare requirements. Based on size, larvae were removed from sheep of the treated groups after 48- and/or 72-h. Often only a subsample of the living larvae was collected from an implant site, especially if there were large numbers. Once removed, the larvae of each strain were placed in individual containers, for that sheep, which contained vermiculite. These were labelled with the strain name, treatment group, sheep number, date and hours post-implant and left to pupate at 27 °C. After the number of pupae and flies which successfully eclosed were counted and recorded, the implant was declared positive if a single fly, or more, eclosed otherwise it was declared negative. A positive implant of a strain was considered a protection failure by the product on that sheep. A protection failure cut-off was set at three sheep per group for the declaration of a break in the protection period, which was confirmed by the subsequent implant. Implants ceased on a treatment group when a break was confirmed or when the products' protection period was reached.

The site of each implant was clipped and swabbed with tincture of iodine, at either 24, 48 and/or 72 h post implant, as warranted. This process successfully killed any remaining larvae and aided the drying and healing process. In addition, the implant or struck areas were re-checked prior to the sheep returning to their runs. Throughout the study sheep were also monitored for naturally occurring flystrikes as there was flystrike activity amongst other flocks of sheep on the property.

The timetable for implant occasions 1 to 10 was designed to accommodate each products' registered protection period. Implants were performed at 2 weekly intervals, except for implant occasions 8–10, which were carried out at 3 weekly intervals (Table 1).

**Table 1**

The *in-vivo* challenge timetable developed to accommodate the flystrike prevention label claims of the products assessed in this study.

Implant Occasions	Weeks Post-Treatment						Untreated Controls (Implant Number)
	Spray-On Formulations			Hand Jetted			
	12.5 g/L Dicyclanil	50 g/L Dicyclanil	65 g/L Dicyclanil	500 g/L Cyromazine	16.0 g/L Ivermectin		
1	3	4	7	3	4	1	
2	5	6	9	5	6	2	
3	7	8	11	7	8	3	
4	9	10	13	9	10	4	
5	11	12	15	11	12	5	
6	13	14	17	13	14	6	
7		16	19	15		7	
8		19	22			8	
9		22	25			9	
10		25	28			10	

### 2.5. Weather conditions

Rainfall, temperature and relative humidity were logged at the experimental site using an automated 'Junior' weather station (Measurement Engineering Australia, Magill, SA, Australia). Whilst data were collected automatically at a frequency of every 10 min, the 24hr totals recorded at 9am daily were downloaded manually. Data were collected from the time of first shearing, at the beginning of August (August 8, 2018), until the sheep were placed back into their runs following their final implant at the beginning of April (6.4.2019). This trial was conducted throughout the main flystrike seasons of spring through to autumn, with implants commencing at the start of November (5.11.2018).

During this study 601.6 mm of rain was received, however, sheep were moved in their groups to the shed to avoid wetting. There were three rain event exceptions (Fig. 1) when the sheep which were not involved in implant occasions 3, 6 or 7 were not moved to a shed, however, they could seek the shelter in their runs. A total of 2.6, 4.2 and 7.8 mm of rain fell on these three occasions respectively. During the final days of August, the lowest minimum temperature was recorded as -2.9 °C. Overall, January was the warmest month on record, up to that time, with the highest maximum daily temperature recorded as 41 °C. The relative humidity ranged from 0.2% to 100%, with an average of 37.8%, over the trial period.

### 2.6. In-vitro assays

The responses of the DSus, DRes4 and DRes8 strains to dicyclanil were determined at the commencement of the trial and 241 days later following the final implant using a previously described bioassay technique (Levot and Sales, 2004). These dose mortality data were corrected for control mortality using the Schneider-Orelli's formula (Püntener, 1981) which is an adaption of Abbotts Correction (Abbott, 1925). Probit analysis (Finney, 1971) was performed using BioStatPro software (BioSat: AnalystSoft) to calculate the LC50 and LC95 values and the associated 95% fiducial limits. The values obtained for DRes4 and DRes8 were compared to that of DSus to estimate resistance ratios.

## 3. Results

### 3.1. *Lucilia cuprina* strains

The LC50 and LC95 values calculated for the dicyclanil susceptible and resistant strains increased over the course of the trial (Table 2). The resistance ratio (RR) calculated at the LC50 relative to the DSus strain, increased 2.4-fold for DRes4 and 1.8-fold for DRes8. As the LC95 values of DRes4 and DRes 8 had overlapping 95% fiducial limits there had not been a significant change in this parameter over the study period. In

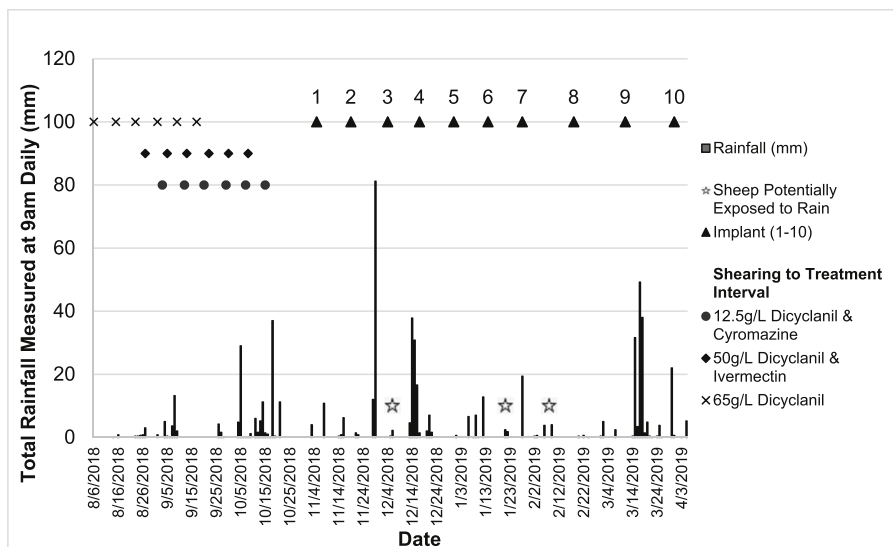


Fig. 1. The daily rainfall (mm) across the study period in relation to shearing, treatment and implant occasions.

contrast, the 95% fiducial limits did not overlap on the DRes4 and DRes8 LC50 values nor the LC50 and LC95 values for the DSus strain.

3.2. Larval implants

The veracity of a cut-off, set at three protection failures out of the six sheep challenged, to declare a break in protection was tested. The upper 95% confidence limit for the expected number of protection failures from six sheep, given observed protection failures ranging from 1 to 5, are shown in Table 3. The 3/6 protection failure cut-off was close to optimum in that a lower cut-off rule (only 1 or 2 protection failures) offered less convincing evidence that the treatments protection was broken against that strain, whereas a higher cut-off rule (4 or more sheep with a protection failure) would be considered excessive and unnecessary.

The low concentration dicyclanil spray-on product (12.5 g/L dicyclanil) failed to halt the development of 347 DRes larvae into flies. These had been collected from all six of the sheep at implant occasion 1 (Fig. 2a). This was three weeks post treatment and represented a > 73% reduction in the period of protection. The 50 g/L dicyclanil group displayed a ≥78% reduction in protection period, four weeks post-treatment, when 45 flies developed from the DRes larvae collected off three of the six sheep at implant occasion 1 (Fig. 2b.) The high concentration dicyclanil based product (65 g/L) had 207 DRes flies eclose from larvae removed from the six implanted sheep at implant occasion 2 (Fig. 2c). As this was only nine weeks post-treatment it equated to a 69% reduction of the protection period. The cyromazine based jetting fluid provided protection to all six sheep on implant occasions 1 and 2. However, seven weeks post treatment, at implant occasion 3, all six implanted sheep had protection failures (Fig. 2d), with 337 flies eclosed. This was a 50% reduction in protection by cyromazine against

Table 2

The *in vitro* response of dicyclanil susceptible and resistant strains of *Lucilia cuprina* to dicyclanil prior to commencement and following completion of the study.

Strain	Study	LC50	(95%FL)	Resistance Ratio	LC95	(95%FL)	Resistance Ratio
DSus	Pre	0.0222 <sup>a</sup>	(0.0209–0.0235)	–	0.0439 <sup>b</sup>	(0.0398–0.0498)	–
	Post	0.0262 <sup>a</sup>	(0.0243–0.0279)	–	0.0580 <sup>b</sup>	(0.0519–0.0672)	–
DRes4	Pre	0.2969 <sup>c</sup>	(0.2333–0.3808)	13.4	1.0360	(0.6968–2.3074)	23.6
	Post	0.8505 <sup>c</sup>	(0.7885–0.9194)	32.5	2.3435	(1.9832–2.9450)	40.4
DRes8	Pre	0.5631 <sup>d</sup>	(0.4036–0.7840)	25.4	1.5818	(1.0403–4.7594)	36.0
	Post	1.2196 <sup>d</sup>	(0.8364–1.6878)	46.5	2.3075	(1.6737–10.3442)	39.8

a, b, c, d 95% Fiducial Limits do not overlap.

Table 3

Verification of the cut-off rule of 3 out of 6 sheep of a treatment group with protection failures against a single strain. This rule was used to define a “break” in the protection period of each flystrike prevention product.

Observed number of protection failures per strain, per treatment group.	Upper 95% confidence limit.
1	3.5
2	4.4
3	5.1
4	5.6
5	5.9

the DRes strain. The ivermectin jetting product had a break in protection declared after the 3rd implant occasion, eight weeks post treatment. A total of 223 flies developed from the DRes larvae removed from five of the six sheep implanted (Fig. 2e) which represented a 33% reduction in the protection period. All products met or exceeded the claimed protection periods against challenge by DSus when the 3/6 protection failure cut-off rule was applied. However, there were some individual sheep protection failures (Fig. 2a–e). The success of the implant challenge technique was demonstrated by all six untreated control sheep having positive implants to both strains on every implant occasion. The average number of eclosed flies, per implant occasion, from all six untreated control sheep was 473 for DRes and 435 for the DSus strain (Fig. 2f). Unlike the DSus strain, implants with the DRes strain ceased on the untreated control group after implant occasion 4, as breaks in protection had been declared on every treated group.

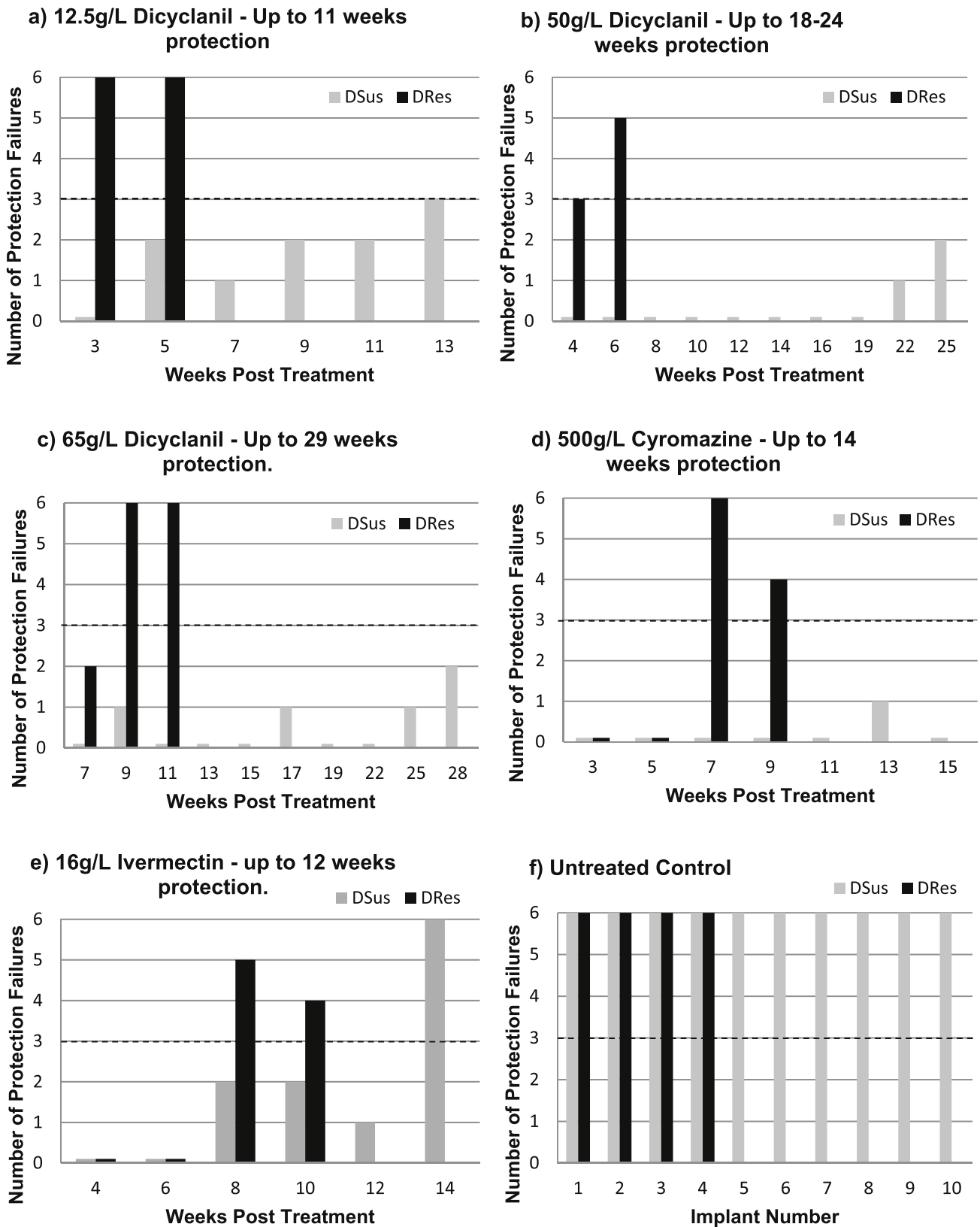


Fig. 2. The number of protection failures, based on adult emergence, following implantation with dicyclanil susceptible and resistant neonate *Lucilia cuprina* larvae. Treatments included a) 12.5 g/L dicyclanil, b) 50 g/L dicyclanil and c) 65 g/L dicyclanil spray-on products; d) cyromazine and e) ivermectin jetting fluids; and f) untreated controls. Also listed is the maximum protection period claimed by each product as weeks post-treatment.

#### 4. Discussion

We report for the first time on reduced protection periods of dicyclanil based spray-on products due to dicyclanil resistance in the Australian sheep blowfly, *L. cuprina*. Additionally, we found that cyromazine, a chemical belonging to the same group, and ivermectin, an unrelated chemical, provided shorter periods of protection against flystrike by dicyclanil resistant larvae when compared to that achieved against a pooled dicyclanil susceptible field strain.

A survey of Australian *L. cuprina* populations, conducted between 2012 and 2014, found 14% of submissions were resistant to cyromazine and dicyclanil, when assessed *in vitro* (Levot, 2014). As the information provided by NSW submitters in 2017–2018 specified historical use of cyromazine and current, exclusive, long-term use of dicyclanil for the prevention of flystrike it was not unexpected when each of the ten *L. cuprina* samples from NSW displayed cyromazine resistance. However, it was concerning that they also proved to be dicyclanil resistant and nine of them were able to survive 4 or 8-fold the dicyclanil susceptible discriminating concentration (SDC). These findings supported the general concerns expressed by some submitters regarding reduced flystrike protection from one or both of these insecticides.

Dicyclanil belongs to the insect growth regulator (IGR) group of chemicals as does cyromazine. The precise mode of action of dicyclanil is unknown, however, it is thought to act in a similar manner to cyromazine by interfering with moulting and pupation (Friedel and McDonnell, 1985). In laboratory studies, dicyclanil was found to be approximately ten times more potent than cyromazine (Graf, 1993), which was released in Australia to prevent flystrike in 1979 (Hart et al., 1979). Despite a report in 2001 (Levot, 2001) that several producers believed that protection periods provided by cyromazine had decreased, it took a further 11 years before cyromazine resistance was confirmed (Levot, 2012). The use of cyromazine for approximately 32 years prior to resistance being described in the field suggests cyromazine resistance in *L. cuprina* is the result of *de novo* resistance evolution (Hawkins et al., 2019).

In 1999, a dicyclanil based spray-on product was released in Australia which had been demonstrated to prevent flystrike for a period of at least 20 weeks (Bowen et al., 1999; Hyman et al., 2000). Three spray-on products of varying concentration are currently available with protection periods claimed from 11 up to 29 weeks ([www.Elanco.com.au](http://www.Elanco.com.au)). These long periods of protection have ensured the rapid uptake and extensive use of dicyclanil based products across Australia to prevent flystrike.

Historically, body strike was considered the most concerning form of flystrike for producers as management practices such as crutching, ringing and surgical procedures like mulesing and pizzle dropping were seen as capable of minimising other types of strikes (Wardhaugh et al., 2001). The move away from mulesing and the selection of more flystrike resistant sheep have shifted emphasis onto breech strike. The breech is particularly problematic and highly variable because of soiling from urine and faeces which ensure attractive oviposition sites and warrants the application of additional insecticide. The variability of the breech was demonstrated by a study comparing mulesed sheep with unmulesed sheep treated with dicyclanil (50 g/L) (Larsen et al., 2012). Only 10–12 weeks protection was obtained against naturally occurring breech strike compared to the 18–24 weeks expected. This lead Larsen et al. (2012) to speculate that protection claims were determined on mulesed not unmulesed sheep. As dicyclanil based products can be applied to any length wool another variable to consider is wool length. An earlier study (James et al., 2009) considered wool length on the breech and found 30–20% of breech implants were positive on sheep 12 weeks post treatment with dicyclanil (50 g/L) unless applied 6 weeks off shears. In the study reported here, assessment on the body rather than the variable breech area and treatment 6 weeks off shears were specifically selected to provide optimal opportunity for flystrike treatment activity. This also allowed comparison of the effect of dicyclanil

resistance on protection periods with studies conducted on cyromazine resistance where body strike was also studied (Baker et al., 2014) and implants were placed within the treated area (Levot et al., 2014). Several other study parameters were modified. Firstly, Levot, et al. (2014) questioned the translocation of cyromazine to skin level when sheep were treated in 7 months wool. The use of cyromazine jetting fluid, rather than the spray-on product, on sheep 6 weeks off shears ensured cyromazine was delivered to skin level in this study. Secondly, we ensured chemical wash out did not occur by housing animals during precipitation and grazing outside for the remainder of the trial. There were three exceptions to this when the sheep which were not being implanted did not get moved into a shed. On the first occasion a total of 2.6 mm of rain fell over 2 days during implant occasion 3. Rain could have fallen on the sheep in their runs if they did not seek shelter. Cyromazine is water soluble and is known to move down the wool staple after small volumes of rain (Hart et al., 1982). This may account for the slight increase in cyromazine efficacy we observed at implant occasion 4. To a lesser extent, an increase in efficacy was also observed in the ivermectin jetted group despite the product claiming to be unaffected by moderate rainfall ([www.coopersanimalhealth.com.au](http://www.coopersanimalhealth.com.au)). In contrast we did not observe any rain associated effects on the dicyclanil spray-on treated groups in line with product claims of Rain Lock™ or Fleece-Bind™ technology ([www.elanco.com.au](http://www.elanco.com.au)).

Dicyclanil is not water soluble and the flystrike products are “suspensions” of dicyclanil dissolved in lipid, non-polar dispersing agents and a dye. Once applied, the dicyclanil dissolves into the lanolin and suint, spreading from the tip of the fleece to skin level while also moving around the animal to include the belly wool (Hosking et al., 2001) and onto new wool growth (Elanco, 2019). This movement of dicyclanil has been called “rapid diffusion” by one manufacturer ([www.jurox.com.au](http://www.jurox.com.au)) and was supported by James et al. (2009) who found dicyclanil transferred from treated breeches to untreated breeches when sheep were run as a single flock. In addition, residue studies in merinos treated 6 weeks off shears with approximately twice the dose rate (100 mg/kg of bodyweight) found dicyclanil and its metabolite in muscle, liver, kidney and renal fat when tested 11 days after treatment (European Agency for the Evaluation of Medicinal Products. Veterinary Medicines and Information Technology, 2000). Most importantly, in the current study the criteria for treatment failure was the development of larvae removed from the sheep through to flies, rather than subjective assessment of larval survival on the sheep. This provided definitive information regarding the viability of the exposed larvae and the product's protective period.

This study included a positive treatment control group, an ivermectin based jetting fluid, which belongs to the macrocyclic lactone (ML) chemical group. The mode of action of ivermectin is very different to that of cyromazine and dicyclanil as it causes paralysis of the insect. This occurs when ivermectin binds to the ligand gated chloride channels which produces ion influx and results in inhibition of nerve impulses (Laing et al., 2017). Ivermectin based flystrike products claim to prevent existing flystrike for up to twelve weeks but only under low to moderate fly pressure ([www.coopersanimalhealth.com.au](http://www.coopersanimalhealth.com.au)). In this study, 12 weeks protection was achieved by the ivermectin product against the dicyclanil susceptible strain (DSus), while only eight weeks protection was attained against the dicyclanil resistant strain (DRes). Increased tolerance, or cross resistance, to cyromazine and/or dicyclanil has been previously reported in strains resistant to other insecticides. When high level resistance developed in *L. cuprina* to the IGR diflubenzuron (Sales et al., 2001), cross resistance was reported to dicyclanil, cyromazine and ivermectin (Levot and Sales, 2004, Batterham et al., 2006). Further studies of these organophosphate and diflubenzuron resistant strains also demonstrated reduced efficacy of registered flystrike dressings containing either ivermectin or spinosad (Levot and Sales, 2008).

Dicyclanil resistance was identified in *L. cuprina* populations which were cyromazine resistant whilst cyromazine resistance occurred

independently (Levot, 2014). This suggests dicyclanil resistance requires the standing genetic variation provided by cyromazine resistance. Cyromazine resistance was shown by bioassay to be incompletely dominant in the sheep blowfly (Levot, 2012) as was the dicyclanil resistance reported here (Sales and Suann unpublished data). As a result, the window of selection is narrower than if either of the resistances were completely dominant (South et al., 2019). However, as the insecticide degrades, the proportion of the population surviving within this selection window at any given time will be greater as it will also include heterozygotes.

It may be speculated that the shorter protection period (11 weeks) provided by the lower dose dicyclanil flystrike product could have expedited the development of dicyclanil resistance in *L. cuprina*. This product could degrade to selective concentrations on sheep while there is still flystrike activity if the product is not used strategically prior to shearing, crutching or the onset of weather conditions unsuitable for flystrike, such as cold winters or hot dry summers. The manufacturers' instructions stipulate its use for short term protection of sheep intended for slaughter, lambs following marking or for protection late in the fly season ([www.elanco.com.au](http://www.elanco.com.au)). However, the opportunity for selection would be increased if these instructions were not adhered to, if treated sheep were not shorn before the dicyclanil decayed to selective levels and blowfly pressure was present, a lower dose than stipulated was applied, or if resistance was already present. Conversely, it could be argued that the 29-weeks protection provided by the high dose (65 g/L) dicyclanil product would reduce selection pressure if the dicyclanil levels remain high and only decrease to selecting concentrations when flystrike pressure was very low or not present at all. This may not be the case if it is applied at the beginning of spring in areas where favourable weather conditions for *L. cuprina* extend from the beginning of spring through to the end of autumn, a total of 39 weeks, or if resistance is already present.

Dicyclanil resistance will have far reaching effects on the management of sheep by Australian producers across the production year. Producers may no longer be able to solely rely on a single treatment with dicyclanil (up to 29 weeks protection) or cyromazine (up to 14 weeks protection) based products to protect sheep across an entire fly season. Sheep producers are encouraged to investigate other non-chemical options which includes mulesing, breeding sheep that are less prone to flystrike and identifying the highest risk periods for flystrike. By using strategic timing of drenching, crutching and shearing, minimum wool and/or a clean breech can be achieved going into high risk flystrike periods. In areas with only low levels and frequencies of dicyclanil resistance, producers may still have the opportunity to decrease selection pressure and possibly prolong the effective life of these insecticides. Therefore, implementation of an integrated resistance management plan, based on rotation of the insecticide group used for the prevention of flystrike is recommended. To be widely adopted any resistance management plan must be cost effective, adaptable across the climatic conditions of the sheep producing areas of Australia and be flexible around the activities of mixed agricultural enterprises such as cropping.

There are other insecticides registered for flystrike prevention in Australia such as spinosad, imidacloprid and ivermectin. Products based on these actives provide much shorter periods of protection and can be used effectively, for example, in the latter stages of the fly season or prior to crutching or shearing. However, the impact of dicyclanil resistance on the efficacy of these alternative insecticides warrants further attention.

With increasing animal welfare concerns (RSPCA Research Report, 2019) and the possible loss of these extensive periods of protection, there is little human or economic tolerance for flystrike amongst Australian flocks. Therefore, it is imperative that the information (Horton and Hogan, 2010) and Flyboss decision-making tools (FlyBoss Tools; <http://www.flyboss.com.au>) which are available on flystrike and insecticide resistance management are utilized by Australian sheep producers.

## Statement of compliance

Mention of Trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by NSW Department of Primary Industry.

Chemicals used in this trial were commercially available registered flystrike preventative products which were purchased from a retailer.

This work was conducted with the approval and oversight of the Animal Ethics Committee and Animal Research Review Panel according to the Animal Research Regulation 2010 of NSW and the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition, 2013).

## Acknowledgements

**Funding:** This work was supported by Australian Wool Innovation (AWI) [grant number ON-00491] and NSW Department of Primary Industries. AWI is grateful for its funding, which is primarily provided by Australian woolgrowers through a wool levy and by the Australian Government which provides a matching contribution for eligible R&D activities.

**Acknowledged Individuals:** The authors thank Dr Peter James (Queensland Alliance for Agriculture and Food Innovation) and Dr Brian Horton (University of Tasmania) for critically evaluating the trial protocol. The following NSW Department of Primary Industries staff are thanked for generously providing:

Sheep and shearing - Greg Glasgow, Farm Manager (Elizabeth Macarthur Agricultural Institute).

Animal feeding and shed cleaning - Shane Koeford, Farm assistant (Elizabeth Macarthur Agricultural Institute).

Biometric advice on expected protection failures - Stephen Morris, Biometrician, (Chief Scientists Branch).

Assistance with the figures - Blake Brangwin, Technical Officer (Elizabeth Macarthur Agricultural Institute).

Early versions of this manuscript were kindly reviewed by Dr Grant Herron, Research Officer Plant Biosecurity (Elizabeth Macarthur Agricultural Institute) and Dr Sue Mortimer, Research Scientist (Armidale Livestock Industries Centre).

## References

- Abbott, W., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 265–267.
- Baker, K., Rolfe, P., George, A., Vanhoff, K., Kluver, P., Bailey, J., 2014. Effective control of a suspected cyromazine-resistant strain of *Lucilia cuprina* using commercial spray-on formulations of cyromazine or dicyclanil. *Aust. Vet. J.* 92 (10), 433–437. <https://doi.org/10.1111/avj.12247>.
- Batterham, P., Hill-Williams, A., Levot, G., Sales, N., McKenzie, J., 2006. The genetic bases of high-level resistance to diflubenzuron and low-level resistance to cyromazine in a field strain of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: calliphoridae). *Aust. J. Entomol.* 45 (1), 87–90. <https://doi.org/10.1111/j.1440-6055.2006.00504.x>.
- BioStatAnalystSoft Inc Statistical Analysis Program. See. <http://www.analystsoft.com/en/>, Version v7, Accessed date: 1 August 2019.
- Bowen, F., Fisara, P., Junquera, P., Keevers, D., Mahoney, R., Schmid, H., 1999. Long-lasting prevention against blowfly strike using the insect growth regulator dicyclanil. *Aust. Vet. J.* 77, 454–460. <https://doi.org/10.1111/j.1751-0813.1999.tb12092.x>.
- Bureau of Meteorology, [http://www.bom.gov.au/climate/averages/tables/cw\\_068192.shtml](http://www.bom.gov.au/climate/averages/tables/cw_068192.shtml), Accessed date: 1 August 2019.
- Coopers Animal Health, <http://www.coopersanimalhealth.com.au/products/CoopersBlowflyAndLiceJettingFluid>, Accessed date: 1 August 2019.
- Elanco, 2019. The Elanco™ Blowfly Control Range. <https://cdn.agriland.ie/uploads/2019/06/Elanco-Blowfly-Detailer.pdf>, Accessed date: 1 August 2020.
- European Agency for the Evaluation of Medicinal Products, Veterinary Medicines and Information Technology, March 2000. Committee for Veterinary Medicinal Products. Dicyclanil. Summary Report (2). EMEA/MRL/733/00-FINAL. [https://www.ema.europa.eu/en/documents/mrl-report/dicyclanil-summary-report-2-committee-veterinary-medical-products\\_en.pdf](https://www.ema.europa.eu/en/documents/mrl-report/dicyclanil-summary-report-2-committee-veterinary-medical-products_en.pdf), Accessed date: 8 January 2020.
- Finney, D., 1971. *Probit Analysis*, third ed. Cambridge University Press, London.
- FlyBoss Tools, <http://www.flyboss.com.au/sheep-goats/tools.php>, Accessed date: 1 August 2019.
- Friedel, T., McDonnell, P.A., 1985. Cyromazine inhibits reproduction and larval

- development of the Australian Sheep Blowfly (Diptera: calliphoridae). *J. Econ. Entomol.* 78 (4), 868–873. <https://doi.org/10.1093/jee/78.4.868>.
- Graf, J., 1993. The role of insect growth regulators in arthropod control. *Parasitol. Today* 9, 471–474. [https://doi.org/10.1016/0169-4758\(93\)90106-](https://doi.org/10.1016/0169-4758(93)90106-).
- Hart, R., Cavey, W., Ryan, K., Moore, B., Strong, M., 1979. Technical details of a new sheep blowfly insecticide. *Wool Technol. Sheep Breed.* 27, 23–27.
- Hart, R., Cavey, W., Ryan, K., Strong, M., Moore, B., Thomas, P., Boray, J., von Orelli, M., 1982. CGA-72662 - a new sheep blowfly insecticide. *Aust. Vet. J.* 59 (4), 104–109. <https://doi.org/10.1111/j.1751-0813.1982.tb02741.x>.
- Hawkins, N., Bass, C., Dixon, A., Neve, P., 2019. The evolutionary origins of pesticide resistance. *Biol. Rev.* 94, 135–155. <https://doi.org/10.1111/brv.12440>.
- Holdsworth, P., Vercruyse, J., Rehbein, S., Peter, R., De Bruin, C., Letonja, T., Green, P., 2006. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of ectoparasiticides against myiasis causing parasites on ruminants. *Vet. Parasitol.* 136, 15–28. <https://doi.org/10.1016/j.vetpar.2005.11.007>.
- Horton, B., Hogan, L., 2010. FlyBoss: a web-based flystrike information and decision support system. *Anim. Prod. Sci.* 50 (12), 1069–1076. <https://doi.org/10.1071/AN10093>. <http://www.publish.csiro.au/AN/AN10093>.
- Hosking, B., Stevenson, C., George, B., Smal, M., Neutze, S., Schmid, H., 2001. Company stewardship of sheep ectoparasiticides. In: Proceedings of the FLICS Conference, Launceston, June 2001, . [http://www.licebosstools.org.au/FLICS/PDFs/Chapter\\_4/Hosking\\_et\\_al\\_Company\\_stewardship.pdf](http://www.licebosstools.org.au/FLICS/PDFs/Chapter_4/Hosking_et_al_Company_stewardship.pdf), Accessed date: 1 August 2020.
- Hughes, P., Shanahan, G., 1978. Assessing OP insecticide resistance in sheep blowflies. *The Agricultural Gazette of NSW* 89, 6.
- Hyman, W.B., Junquera, P., De Bruin, C., Van Zyl, A.P., Schmid, H.R., 2000. Flystrike prevention on Merino lambs with the insect growth regulator dicyclanil. *S. Afr. Vet. Ver.* 71 (1), 28–30. <https://www.ingentaconnect.com/content/sabinet/savet/2000/00000071/00000001/art00007>.
- James, P., Cramp, A., Winkleman, J., McPhie, R., Brown, G., 2009. Strategic use of crutching and dicyclanil to protect sheep against breech strike. *Aust. Vet. J.* 87, 138–141. <https://doi.org/10.1111/j.1751-0813.2009.00408.x>.
- Laing, R., Gillan, V., Devaney, E., 2017. Ivermectin – old drug, new tricks? *Trends Parasitol.* 33 (6), 463–472. <https://doi.org/10.1016/j.pt.2017.02.004>.
- Lane, J., Jubb, T., Shephard, R., Webb-Ware, J., Fordyce, G., 2015. Priority List of Endemic Diseases for the Red Meat Industries in: Final Report B.AHE.0010 Prepared for Meat & Livestock Australia. Sydney. <http://www.mla.com.au/Research-anddevelopment/Search-RD-reports/RD-report-details/Animal-Health-andBiosecurity/Priority-list-of-endemic-diseases-for-the-red-meat-industries/2895>, Accessed date: 1 August 2019.
- Larsen, J., Tyrell, L., Anderson, N., 2012. Prevalence of breech-strike in mulesed, clipped and unmulesed Merino hoggets in south-eastern Australia. *Aust. Vet. J.* 90, 158–166. <https://doi.org/10.1111/j.1751-0813.2012.00914.x>.
- Levot, G., 2001. Implications of insecticide resistance for the control of flystrike and lice on sheep. In: Champion, S. (Ed.), FLICS Conference. Tasmanian Institute of Agricultural Research, Launceston, pp. 127–134. [http://webb2.jet.studiocoast.com.au/FLICS/PDFs/Chapter\\_3/Levot\\_Insecticide\\_resistance.pdf](http://webb2.jet.studiocoast.com.au/FLICS/PDFs/Chapter_3/Levot_Insecticide_resistance.pdf), Accessed date: 1 August 2019.
- Levot, G., 2012. Cyromazine resistance detected in the Australian sheep blowfly. *Aust. Vet. J.* 90 (11), 433–437. <https://doi.org/10.1111/j.1751-0813.2012.00984.x>.
- Levot, G., 2014. Resistance to flystrike preventative treatments. In: AWI Breech Strike R&D Technical Update. Maritime Museum, Sydney 20th August 2014. [https://www.wool.com/globalassets/start/on-farm-research-and-development/sheep-health-welfare-and-productivity/sheep-health/breech-flystrike/r-and-d-update/20140820\\_12\\_awi\\_rnd\\_tech\\_update\\_levot.pdf](https://www.wool.com/globalassets/start/on-farm-research-and-development/sheep-health-welfare-and-productivity/sheep-health/breech-flystrike/r-and-d-update/20140820_12_awi_rnd_tech_update_levot.pdf), Accessed date: 1 August 2019.
- Levot, G., Sales, N., 2004. Insect growth regulator cross-resistance studies in field- and laboratory-selected strains of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: calliphoridae). *Aust. J. Entomol.* 43 (4), 374–377. <https://doi.org/10.1111/j.1440-6055.2004.00394.x>.
- Levot, G., Sales, N., 2008. In vitro effectiveness of ivermectin and spinosad flystrike treatments against larvae of the Australian sheep blowfly *Lucilia cuprina* (Wiedemann) (Diptera: calliphoridae). *Aust. J. Entomol.* 47, 365–369. <https://doi.org/10.1111/j.1440-6055.2008.00670.x>.
- Levot, G., Langfield, B., Aiken, D., 2014. Survival advantage of cyromazine-resistant sheep blowfly larvae on dicyclanil- and cyromazine-treated Merinos. *Aust. Vet. J.* 92 (11), 421–426. <https://doi.org/10.1111/avj.12251>.
- Magoc, L., Yen, J., Hill-Williams, A., McKenzie, J., Batterham, P., Daborn, P., 2005. Cross-resistance to dicyclanil in cyromazine-resistant mutants of *Drosophila melanogaster* and *Lucilia cuprina*. *Pestic. Biochem. Physiol.* 81 (2), 129–135. <https://doi.org/10.1016/j.pestbp.2004.11.002>.
- McLeod, J., 1937. The experimental production of cutaneous myiasis in sheep. *Parasitology* 29, 526–529. <https://doi.org/10.1017/S003118200002504X>.
- Püntener, W., 1981. Manual for Field Trials in Plant Protection, second ed. Agricultural Division, Ciba-Geigy Limited OL17686923M. <https://www.random.org/integers/>, Accessed date: 1 December 2019.
- RSPCA Research Report: prevention and control of blowfly strike in sheep. <https://kb.rspca.org.au/wp-content/uploads/2018/11/Research-report-Prevention-and-control-of-flystrike-in-sheep-January-2019.pdf>, Accessed date: 1 August 2019.
- Sales, N., Levot, G., Barchia, I., 2001. Differences in susceptibility to diflubenzuron between populations of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) and their influence on flystrike protection. *Gen. Appl. Entomol.* 30, 27–30. <https://search.informit.com.au/documentSummary;dn=215624873815621;res=IELHSS>, Accessed date: 1 December 2019.
- South, A., Lees, R., Garrod, G., Carson, J., Malone, D., Hastings, I., 2019. The role of windows of selection and windows of dominance in the evolution of insecticide resistance in human disease vectors. *Evol. Appl.* 1–14. <https://doi.org/10.1111/eva.12897>. 00.
- Wardhaugh, K., Mahon, R., Bedo, D., 2001. Factors affecting the incidence of flystrike in Australia. In: Proceedings of FLICS – Flystrike and Lice IPM Control Strategies, pp. 80–87.
- jurox <https://www.jurox.com/au/product/strikeforce-s/formulation>, Accessed date: 10 August 2020.
- Yen, J.L., Batterham, P., Gelder, B., McKenzie, J.A., 1996. Predicting resistance and managing susceptibility to cyromazine in the Australian sheep blowfly *Lucilia cuprina*. *Aust. J. Exp. Agric.* 36, 413–420. <https://doi.org/10.1071/EA9960413>.